Protein-lipid interactions and non-lamellar lipidic structures in membrane pore formation and membrane fusion

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Abstract

Pore-forming proteins and peptides act on their targeted lipid bilayer membranes to increase permeability. This approach to the modulation of biological function is relevant to a great number of living processes, including; infection, parasitism, immunity, apoptosis, development and neurodegeneration. While some pore-forming proteins/peptides assemble into rings of subunits to generate discrete, well-defined pore-forming structures, an increasing number are recognised to form pores via mechanisms which co-opt membrane lipids themselves. Among these, membrane attack complex-perforin/cholesterol-dependent cytolysin (MACPF/CDC) family proteins, Bax/colicin family proteins and actinoporins are especially prominent and among the mechanisms believed to apply is the formation of non-lamellar (semi-toroidal or toroidal) lipidic structures. In this review I focus on the ways in which lipids contribute to pore formation and contrast this with the ways in which lipids are co-opted also in membrane fusion and fission events. A variety of mechanisms for pore formation that involve lipids exists, but they consistently result in stable hybrid proteolipidic structures. These structures are stabilised by mechanisms in which pore-forming proteins modify the innate capacity of lipid membranes to respond to their environment, changing shape and/or phase and binding individual lipid molecules directly. In contrast, and despite the diversity in fusion protein types, mechanisms for membrane fusion are rather similar to each other, mapping out a pathway from pairs of separated compartments to fully confluent fused membranes. Fusion proteins generate metastable structures along the way which, like long-lived proteolipidic pore-forming compexes, rely on the basic physical properties of lipid bilayers. Membrane fission involves similar intermediates, in the reverse order. I conclude by considering the possibility that at least some pore-forming and fusion proteins are evolutionarily related homologues.

Keywords: membrane pore formation, non-lamellar (toroidal) lipids, membrane fusion, membrane fission, molecular evolution

1. *Biological membranes: barrier and gateway*

Biological membranes represent both challenges and opportunities. They represent challenges because they seal the outside of the cell and provide for interior compartments within cells, presenting a barrier to the transfer of nutrients and signals. They represent opportunities because they provide protection, and enable the planar localisation of biological processes and signal transduction [1, 2]. The specificity with which the challenges represented by membranes are met, and with which the opportunities they provide are realised, depends on proteins which mediate the interactions they undergo and which shape, remodel and open channels through their constituent lipid bilayers. But the lipids themselves also play a critical role in enabling membrane biology, providing a structure or framework to processes alongside the proteins driving them. Indeed, because of the presence of a diversity of lipid forms (e.g. those promoting positive and negative curvature, sterols, glycolipids and so on) and organisations (bilayer asymmetry and lateral segregation into rafts) membranes present a complex terrain or landscape within which membrane-acting proteins must operate [3-6]. This review focuses principally on the direct use which two classes of membrane-targeting proteins, pore-forming proteins and fusion proteins, make of bilayer lipids in carrying out their functions. Membrane fission is also considered, and its essential reversal of the fusion process.

Membrane pore formation and membrane fusion/fission are related phenomena. Both involve the remodelling of the membrane bilayer, whereby the pre-existing lipid matrix is perturbed either to open a water-filled channel across it or to bring about its fusion with another membrane-bound compartment of the generation of two compartments from one. It is now becoming generally accepted that both involve lipids as critical components of their mechanism, rather than simply remodelling them in a passive manner [7-9]. And both involve a dramatic refolding of the principal actors, the pore-forming and fusion/fission proteins themselves. Gianfranco Menestrina played a

seminal role in establishing that lipids are active players in membrane pore formation [10-13] and this review is a tribute to his contribution to the establishment of the field under discussion.

2. *Pore-forming proteins*

Pore-forming proteins are typically produced as soluble monomers or dimers which then bind to membranes before self-assembling into a pore-forming structure, usually a complete or incomplete ring of subunits (Figure 1) [14]. Although many or most pore-forming proteins are thought to be monomeric in solution [14], some significant examples are dimeric, including proaerolysin [15] and perfringolysin [16-18]. In both these cases the dimers are antiparallel, whereas self-association to form pores involves a parallel arrangement of subunits. It is possible that antiparallel dimerization is a mechanism for preventing premature oligomerisation, which just depends on (active) protein concentration [16, 19, 20]. Incomplete rings or non-ring-shaped structures such as those formed by membrane attack complex-perforin/cholesterol-dependent cytolysin (MACPF/CDC) family proteins and Bax-like proteins have a more obvious direct role for lipids in pore structure [7, 12, 14, 21-23]; but even where complete rings of subunits form, lipids are now thought to be capable of playing a critical role in how pore formation occurs [24] (See below, Figure 2). Pore-forming proteins have been extensively reviewed in the literature [14, 25, 26] and broadly-speaking fall into two classes: those which use a transmembrane structure made of β strands and those which use a structure made of α helices. In both cases there may be a transition from one form of secondary structure to the other during the pore-formation process – for example CDCs transition into the membrane with the refolding of two sets of α helices into a pair of transmembrane β hairpins [27, 28], whereas the *E. coli* cytolysin A (ClyA, also known as HlyE) makes the opposite journey, and converts some regions of β sheet alongside some loop regions into α helix [29]. Similar pore-forming structure, or mechanisms of pore formation, can also have different origins – for example the *Staphylococcus aureus* α-hemolysin builds a 14-stranded pore by the unfolding of a β hairpin from the β-sandwich structure of each of seven oligomerised subunits [30-33], whereas the *Bacillus anthracis* protective

antigen (PA) forms a barrel with the same number of strands (albeit one of twice the height) from polypeptide regions found as a Greek key motif and exposed loop within monomeric and pre-pore forms of the protein [34, 35].

The paradigm systems for studying membrane targeting by pore-forming proteins have been from bacterial species, and among the most completely understood are the families of proteins related to *S. aureus* α-hemolysin [24, 30] and the MACPF/CDC family of proteins [36, 37]. In a sense these represent opposite ends of a spectrum of pore-forming proteins based on membrane-inserted β-barrel structures. For example, the pores formed by *S. aureus* α-hemolysin and its relatives are small (1-2nm across in functional diameter) since they are composed from just a few subunits (6-8, generally) and are always complete [30-33]. By contrast, pores formed by MACPF/CDC proteins are up to ~30nm across with up to ~40 subunits, with incomplete rings of subunits (ie arcs of subunits) a frequently-observed phenomenon which recent data have shown to be *bona fide* pore-forming complexes [38-40] (Figure 2), in agreement with a long-proposed argument [14, 16, 36, 41, 42] and much functional data [43-47].

This review is structured to consider in turn pore-forming proteins from the β-sheeted and α-helical classes, and within each class to begin with an example of a discrete protein-only pore-forming structure which sits across its target bilayer before looking into pore formation with protein-induced non-lamellar lipid structures, and pore formation by lipids alone via the formation of a toroidal arrangement of lipids (Figure 1d). I then turn to the fusion and fission proteins as an alternative group of membrane-targeting proteins before considering the possible evolutionary relationship between proteins engaged in pore formation and membrane fusion. As a first step, however, I will consider the simplest kinds of pore-forming protein: peptides which permeabilise membranes.

3. *Starting simply: pore-forming peptides*

Small peptides are a well-known form of membrane-targeting agent [7], among which examples include the β-sheet peptides arenicin, protegrin-1 and Alzheimer's β1-41 [48-50] and the α-helical peptides alamethicin and melittin [51]. A variety of possible mechanisms for pore-forming peptides have been advanced, including the formation of complete rings of subunits (the "barrel-stave" model), and the induction of non-lamellar structures by peptides lying either on the surface of or across target membranes [52]. Intriguingly, although alamethicin and melittin have similar amphipathic structures, they actually form pores by distinct mechanisms: in the case of alamethicin with a barrel-stave pore, in the case of melittin by the induction of a non-lamellar phase [51]. However, an interesting difference exists between α-helical and β-sheeted pore-forming peptides as the former are hydrogen-bonding self-sufficient in their interactions with nearby amino acids, whereas β-hairpins must form hydrogen bonds (or possibly another kind of stabilising interaction) with either non-adjacent residues, other polypeptide chains or other species. This distinction affects the pore-forming mechanisms which α-helical and β-sheeted peptides can adopt. While an α-helical peptide might easily lie along a membrane surface, a β-hairpin is unlikely to be able to do so, forcing a trans-bilayer orientation. Furthermore, while orientation across the bilayer might similarly work for a single α-helix – whether as a hydrophobic integral membrane protein or pore-forming peptide – this will not be a tenable location for an isolated β-hairpin. Instead, it must form lateral interactions – whether with at least one other copy of itself or with other species. Indeed, we see this in integral membranes themselves, where the standard orientation of an α-helix is across the membrane and the standard arrangement of a β-strand is as part of a β-barrel.

The essential properties of pore-forming peptides suit them to form pores which make bilayer lipids an inherent component of their structure using either a "matrix-type" approach in which lipids and proteins alternate, or else an "arc-type" mechanism in which the protein clusters on one side of the structure and the lipid (in a non-lamellar arrangement) on the other (Figure 1c) [7]. The arc-type

mechanism can obviously be a sub-form of a full ring of protein subunits. Melittin classifies as an α-helical peptide with a matrix-form assembly mechanism, and arenicin is a β-sheeted peptide which is also thought to have a matrix-type pore arrangement [48]. Evidence from AFM and molecular dynamics simulations indicates that protegrin-1 [49, 53] and Alzheimer's β1-41 peptide [50, 54, 55] form ring-like and arc-based pore structures in target membranes. The formation of pores by arc-shaped assemblies of peptides echoes the equivalent structures formed by pore-forming proteins of the MACPF/CDC class [7].

4. α-hemolysin family proteins – a peripheral role for lipids

S. aureus α-hemolysin was the first pore-forming protein to have its atomic structure solved in a pore-forming state [30] (Figure 1a). This revealed its heptameric assembly and 14-stranded β-barrel. Other studies have shown that this protein assembles as a pre-pore structure first, and then refolds into a pore [56, 57], an influential insight which has had a significant impact on how pore-forming proteins tend to be understood. Indeed, many such proteins will form pre-pore complexes before they form pores, but this is not expected to be a genuine universal. For example, the complement membrane attack complex (MAC) is currently thought not to form a pre-pore assembly in the classic sense but, once membrane targeting has occurred via a complex of proteins C5b-9, that pores open and then grow in size as more C9 subunits join the assembly [58-60].

There is some complexity to the pore-forming mechanism of α-hemolysin and related proteins. Some members of the family are bi-component – they have two homologous subunits which pack alternately to generate the pore-forming structure, examples being leukocidin F of the Panton-Valentin endotoxin [61]. This requires an octameric oligomerisation state, the structure of which has also been solved [31]; meanwhile there is in fact quite good evidence for a hexameric version of the α-hemolysin pore itself based on the measurement of a biphasic conductance distribution and

the calculation that this would equate to functional hexamers alongside heptamers [62]. Nevertheless, the limited size of the pore-forming structures formed by this family of proteins does not tolerate the existence of incomplete rings of subunits as a pore-forming mechanism. Indeed, the evidence from real-time imaging of the assembly of individual pores is that the only stable complexes formed are complete heptamers. After subunit binding, random movement of the subunits on the membrane results in the formation of the minimal dimer which has a lifetime of ~50ms before dissociation [63]. However if assembly growth is sufficiently rapid than seven α-hemolysin subunits can find each other in time, stochastically, to generate the pre-pore structure which ultimately transitions to a pore [63]. Studies of this kind have only so far been performed with α-hemolysin itself, out of this family of proteins, but it would seem likely that pore formation via exactly such a mechanism might be obstructively difficult in the case of the bi-component leukocidins due to the need for the protein subunits to find each other in a precise order, and requiring perhaps an initial dimerization followed by the assembly of further dimers in turn. Recent evidence supports this conjecture [32].

A role for lipids in the pore-forming assemblies generated by α-hemolysin and related proteins would seem to be precluded, yet in fact a recent set of experiments with forms of α-hemolysin in which its transmembrane β-barrel forming loops were truncated have shown that, even though too short to span the membrane, this modified kind of barrel still forms pores [24]. The pore-forming structure involved is thought to be a non-lamellar arrangement of lipids, which is a packing re-arrangement in the normal lipid bilayer structure in which a water-filled channel through the membrane is lined by a highly-curved surface formed from the lipid headgroups [7] (Figure 1d). A structure of this kind is thought to be formed during electroporation [7, 64-67] (see below, section 9). It is quite possible that these α-hemolysin data tell us something very interesting about the mechanism of insertion of pore-forming complexes, because they indicate a way in which such complexes could clear the membrane bilayer lipids out of the way as they insert into membranes.

This has long been a puzzle and may indeed be a general solution found also in pore-forming proteins which construct much larger pore-forming assemblies [7, 40]. By getting the lipids to move themselves out of the way via the generation of an inverted phase, pore-forming proteins generating non-lamellar structures can then pre-open up a channel through the lipids for their insertion (Figure 2a). In turn this might imply that the smaller pore-forming structures evolved first to use this mechanism of lipid clearance. Then, pore-forming proteins which generate larger assemblies relying on non-lamellar lipid arrangements induced by arcs of subunits for added functional versatility could have arisen. This would agree with the increased complexity of phenotype conferred by larger pore-forming assemblies; for example, the delivery of granule proteases from natural killer cells and cytotoxic T-lymphocytes into antigen-presenting cells [44] and the intracellular parasitism of bacteria [43, 68]. Proteins from the α-hemolysin family by contrast more simply punch holes to cause leakage of material from cells and trigger internal signalling responses leading to cell death, rather than seeking to programme cell behaviour [44] or enable intracellular growth of bacteria and their direct movement between cells [43, 68].

5. *MACPF/CDC proteins – do lipids close the circle?*

The MACPF/CDC family of pore-forming proteins demonstrates a very direct role for lipids in the formation of pores in biological membranes (Figure 1b). This derives directly from the capacity of these proteins to form stable, pore-forming arcs of subunits which require contributions from an endogenous component of the membrane they are targeting in order to define a perimeter for the pore-forming channel. From the first observation of pore complexes generated by members of this family, by electron microscopy, the existence of incomplete rings (arcs) alongside the complete ring structures was noted [41, 69, 70]. While some (correctly) concluded that this implied an unconventional mechanism of pore formation [71] in which the interface between a protein arc and the lipid membrane defined the pore boundary [14, 36, 45, 72, 73] most researchers in the field

wrote the idea off and considered it, *a priori*, biophysically implausible [74-76]; oddly, some still do [77].

Besides images of arciform complexes apparently forming pores in membranes, obtained by electron microscopy [69, 70, 78, 79], data which indicate that the MACPF/CDC proteins form proteolipidic pores include the variable functional size of the pores [12, 21, 44, 46, 80, 81], the apparent role played by lipids themselves in the functional characteristics of the pores [46], the way in which pores enable the flip-flopping of lipids from one side of a membrane bilayer to another [44, 81] (a typical indication of pore formation via non-lamellar lipid arrangements [82, 83]) and the kinetic mechanism of pore complex assembly whereby the concentration of active monomeric subunits clearly drives the distribution of oligomer size (i.e. how many complete ring-form and incomplete arc-form complexes there are and in what proportions) [14, 16, 38, 42, 84] (Figures 2c and d).

6. Seeing is believing

Structural biology has consistently been at the leading edge of progress in our understanding of pore-forming proteins. Pore-forming assemblies were first identified using electron microscopy [69, 78, 85]; and, repeatedly, the determination of the atomic structure of pore-forming proteins in a soluble [15, 86, 87] or pore-forming [29, 30] state has triggered an enhanced rate of progress in understanding their mechanisms. Maybe it is not surprising, therefore, that the role of incomplete, arc-shaped assemblies of subunits only finally became accepted when they were imaged using cryo-electron tomography [40] and atomic force microscopy [38, 39, 88] (Figure 2b, d and e). In retrospect this cast (and casts [77]) a negative light on the rejection of the non-imaging data for protein arc-based pore formation as implausible.

The suitability of cryo-electron tomography to address this question derives from its capacity to provide 3D visualisation of pores *in situ* within membranes. Thus, an experiment was designed in which a bacterial MACPF/CDC, pneumolysin, was added to cholesterol-containing membranes and the resulting pre-pore and pore-forming complexes imaged [40]. A full range of oligomeric sizes was seen – from arcs of only a few subunits to complete rings – in both the pre-pore and pore-forming states [40]. The chief interest, however, lay in the distribution of membrane associated with pre-pore and pore-forming arcs of subunits. In the pre-pore state there was no opening in the membrane and instead the oligomer simply sat on its surface; in the pore-forming state the pneumolysin oligomer had inserted into the membrane, there was a hole through the bilayer and the open ends of the arc of subunits had stretched between them a lipidic edge which completed the perimeter of the pore [40] (Figure 2b and c). In addition to assembly states clearly either pre-pore or pore, some complete rings appeared to be in the process of transitioning between the two such that the membrane was partly broken and, in agreement, the oligomer of subunits was partly inserted [40] (Figure 3). This suggests that the process of membrane insertion involves a rolling conformational change, allowing lipids within the centre of the ring of subunits to flow back into the rest of the membrane rather than be jettisoned into solution on oligomer insertion (Figure 3). This rolling back would be accompanied by the formation of a non-lamellar (semi-toroidal) lipid edge, as already discussed (Figures 1b, 2a, 2d). Although a subsequent study using AFM suggested that lipid vesicles may indeed be released from the membrane on pore formation [38] (Figure 2d), another study in which the membrane mass was quantitatively measured also strongly supports pore formation by a diverse set of oligomeric assembly sizes and has indicated that lipids are not lost from membranes when pore formation occurs [89]. Different MACPF/CDC proteins may, of course, use different approaches to clear lipids from the centre of pore-forming structures.

Three AFM studies [38, 39, 88] since the cryo-electron tomography study of pneumolysin pores [40] have strongly backed up the insights it provided (Figure 2d and e). In one, by Leung et al. [38],

the CDC suilysin was imaged forming pre-pore and pore complexes on membranes and it was clearly seen that, as in a previous set of AFM experiments from another lab using perfringolysin [84], pore-forming complexes could either be arcs of subunits or complete rings of subunits (Figure 2d). The beauty of Leung et al.'s work was that they clearly resolved the location of the lipid edge too and formulated a model for pore formation by kinetically-trapped arcs of protein subunits, or by complete rings, depending on the concentration of protein available [38] (Figure 2d). The minimum size of pore-forming arc seemed to be five subunits [38]. This model mechanism is a quantitative version of an old one, first proposed in 2002 [14] and argued periodically since [16, 36, 42] (Figure 2c).

In the second recent study using AFM, Podobnik et al. used listeriolysin pores to investigate the role of lipids in its pore formation and the dynamics shown by individual membrane pores [39] (Figure 2e). In a series of time-resolved experiments they were able to show pore formation by incomplete rings of subunits (arcs) and also the dynamic behaviour of arcs which can come together to form larger pore-forming complexes consisting of an apposed pair of, or more, arcs, which can also subsequently separate into smaller pores [39]. The larger "patchwork" pores were especially intriguing in which multiple arcs came together to form a much larger assembly with a resulting much-increased channel size [39] (Figure 2c and e). The particular significance of this work is the way in which it shows the dynamics of pores, which are expected to apply also to the biological context [43, 68], and also the much greater range of pore size observed than previously. Podobnik et al.'s work provides a way to understand how listeriolysin pores could enable the escape of *Listeria* bacteria from endosomes and their movement between adjacent cells [43, 68]. Its direct visualisation of the real-time separation and joining of pore-forming arcs also echoes insights gained for the MACPF/CDC perforin using electrophysiological measurements [46]. More recently similar findings to Podobnik et al have been made in the third time-resolved AFM-based study of listeriolysin [88].

Thus, seeing is believing, and understanding in the research community has changed almost overnight from a rejection of the possibility of a direct role for lipids in pore formation by MACPF/CDC proteins [7] to an enthusiastic embracing of the concept and all that it means in terms of an enhanced understanding of the versatility of pore-forming mechanisms in health and disease. Isolated naysayers remain [77], but the impact of the newly shared understanding of the mechanism of MACPF/CDC proteins on how the scientific community in turn understands their role in health and disease is obvious (see above and [7, 71]). The suggestion that the use of model systems to study pore formation has resulted in artefactual results [77], firstly, undermines also the experiments which some take to show that only full rings are pore-forming; secondly and more fundamentally seeks to undermine the apparently valid basic assumptions of modern biochemistry and molecular biology; and thirdly ignores data showing without doubt the presence of incomplete MACPF/CDC oligomers forming pores in intact cell membranes [44] and even *in vivo* [90].

The MACPF/CDC proteins represent one particular example of how proteins and lipids can come together to generate pore-forming complexes. As discussed above, it may be an evolutionary development of a mechanism of lipid clearance during the insertion of simpler pore-forming complexes such as α-hemolysin, where a similar non-lamellar arrangement of lipids is used in transition to a fully-inserted barrel of subunits (Figure 2a). Like α-hemolysin, fusion proteins, too, make transitory use of a highly-curved lipid surface as they generate a fusion pore *en route* to the confluence of two membrane bilayers. This will be discussed further below.

7. *Soft edges: pores formed by α-helical assemblies*

A fundamental difference between pore formation engineered via β-barrel structures and via α-helical assemblies is that the hydrogen-bonding patterns of α helices are self-sufficient, whereas

those of β-sheets are not [7]. Like with the examples of β-barrel pore formation discussed already, the α-hemolysin family and the MACPF/CDC family of proteins, there is diversity in the ways in which proteins and lipids interface during membrane insertion of α-helical pores. Occupying an equivalent position in this grouping to α-hemolysin within the β-barrel formers is another bacterial pore-forming protein, the Cytolysin A (ClyA) found in *Escherichia coli* and *Salmonella enterica* strains [29, 91]. ClyA forms dodecameric pores and, as with α-hemolysin, there is no indication that they can as well be incomplete as complete rings. The conformational change involved in ClyA transitioning from a pre-pore assembly into a pore-forming one involves its conversion from a bowed bundle of α-helices with a terminal β-sheet hairpin (or "tongue") into a straight up-and-down all-helical structure. The conformational changes necessary to effect this transition can be placed into a hierarchy and thereby into an inferred temporal sequence [29]. The pre-pore state nascent assembly is partly inserted into the membrane bilayer, before an iris-like coordinated change completes pore formation and stabilizes the transmembrane complex [29]. Although there is no evidence for a role for lipids in the structure of ClyA pores, the partial insertion of the proposed pre-pore structure, which appears to be backed up by imaging data, does however suggest the possibility that a transitory non-lamellar (toroidal) arrangement of lipids (Figure 1d) beneath the forming pore complex [7, 24] eases its passage into the membrane, clearing away the bilayer in a mechanism concerted with protein insertion. This would then be equivalent to the effect seen with truncated forms of α-hemolysin and posited as a general mechanism for lipid clearance during pore formation [24] (Figure 2a).

In other cases of α-helix-based pore-forming assemblies, however, lipids are directly implicated in the mechanism of pore formation. A case in point is the actinoporins found in sea anemones, such as sticholysin II and equinatoxin II [10, 92]. These proteins have a preference for or dependence on sphingomyelin as a point of contact on the membrane surface, and a variety of studies have shown that they make use of lipids in their mechanism of pore formation, though this has long been

thought to be according to a distinct arrangement of protein and lipid compared to the MACPF/CDCs. As discussed above, the MACPF/CDCs form pores at protein-lipid interfaces in which the protein constitutes one curved edge to the pore and the lipid completes the perimeter [7]. The actinoporins instead form a matrix arrangement in which lipid and protein components alternate; one study of sticholysin II, using low-resolution electron crystallography for the pore, suggested this might be a tetrameric assembly with lipids filling in the pore channel perimeter in between the protein subunits [92]. A more recent atomic resolution X-ray crystal structure of the pore-forming state of fragaceatoxin C resolved instead an octamer and showed the presence of pore-specific lipids bound to special locations between subunits, indeed plugging gaps in the channel perimeter [93] (Figure 4a). This suggests that the 4-fold symmetry of the sticholysin II pores, reflecting the P4 symmetry of its two-dimensional crystals, masked an underlying octameric symmetry as found in the FraC structure. Indeed, there are eight density maxima around the perimeter of the sticholysin II pore – in the previous model these were treated as two lobes of density for four protein subunits layed on their side [92]; the FraC structure suggests a more accurate interpretation would be of eight subunits rotated through 90° and viewed down their long axis, since that is the orientation of the subunits in the FraC pore [93].

Direct functional evidence for the involvement of lipids in actinoporin pore-forming assemblies came from work with equinatoxin II in which solid-state NMR (SS-NMR) was used to show a non-lamellar arrangement of lipids in the membrane, and the enhancement of cationic selectivity by the inclusion of negatively-charged lipids indicated the presence of such lipids within the perimeter of the pore [10]. In another study, this time using sticholysin II, lipid flip-flop was enhanced by pore formation, which was itself facilitated by lipids with positive curvature [13]. In fact, the FraC crystal structure does not show a typical partially-toroidal arrangement of lipids within the protein-lipid (proteolipidic) matrix which forms the pore's channel; instead (as shown; Figure 4a) the specially located lipids specific to the pore structure remain in a lamellar orientation, but the

lamellar structure is disrupted by pore formation in line with the SS-NMR data and allowing for the exposure of lipid headgroups to the pore lumen [10, 93]. It is possible that this arrangement could directly result in an enhancement of lipid flip-flop, since its formation clearly perturbs the bilayer. Alternatively, if the formation of a torus of lipids is a standard feature of mechanisms of pore formation – as suggested by the work on truncated forms of α-hemolysin discussed above [24] (Figure 2a) – then the flip-flop could equally well be a result of the transient formation of such structures as the actinoporin inserts into its targeted membrane.

8. Bax against a lipid wall

A second class of pore-forming protein in which a pore-forming helical region of a protein engages with the targeted membrane is found in the case of the pore-forming members of the Bax family of proteins. These proteins are best known for their role in apoptosis, where members such as Bax and Bak perforate the mitochondrial membrane to release cytochrome C and other protein factors promoting apoptotic cell death [94]. In turn, homologous proteins without a pore-forming capacity such as Bcl-2 prevent pore formation – and, in the case of Bcl-2 can thereby promote cancer (Bcl = B cell lymphoma). This family of pore-formers includes also bacterial colicin proteins [95] and apolipoprotein L1 (a component of high-density lipoproteins which also plays a role in human immunity to trypanosomes) [96]. The mechanism of pore formation by these proteins is not entirely resolved. What is clear, however, is that a variety of studies have shown a role for lipids in their pore formation and suggested a non-lamellar (partially toroidal) arrangement via the observation of lipid flip-flop [82, 97, 98]. Perhaps the most direct evidence for this is the use of anomalous X-ray diffraction measurements to show directly that the Bax α5 helix induces membrane pore-forming structures in which lipid headgroups are to be found right through the bilayer [99], which can only really be understood due to the formation of a through-bilayer positively-curved lipid edge (Figure 4b and as in Figure 1b). Further molecular details of Bax pore formation remain the subject of debate, however two recent papers have indicated that previous models in which a refolding of the

α-helical bundle constituted by Bax resulted in the insertion of a hairpin of helixes (α5 and α6) [100] are most likely incorrect [22, 101]. Instead, the laying of α5 and α6 [101] or just α5 [22] out parallel to the membrane plane results in the destabilisation of its lamellar structure and induction of the non-lamellar lipidic arrangement (Figure 4b). This mechanism of pore formation is similar to the "carpet" model proposed for bactericidal peptides [102]; for discussion relevant to Bax and colicins see [103], however it is clear the Bax leaves the basic membrane intact indicating that it forms discrete pore structures and does not destabilise the membrane in a generalised way. In any case, all evidence points to the idea that pore formation by Bax and related proteins leads to a non-lamellar-lipid pore, and quite likely one in which the protein contributes only locally to the channel perimeter – as in the MACPF/CDCs – rather than forming a proteolipidic matrix as in the actinoporins. Indeed, a cryo-electron microscopy study of Bax in a lipid nanodisc has provided a visualisation of a pore in which the protein is on one side, leaving free lipid on the other [104]. The unit of assembly to form a pore appears, in any case, to be a dimer of Bax subunits [22, 101], and the structure of this dimer or something close to it has been resolved by X-ray crystallography [23, 105]. Whether pore formation can occur with a single dimer [104] (this paper models a monomer but clearly it should be a dimer) or requires more than one dimer remains to be seen, but clearly larger pores should result from larger numbers of Bax subunits being present [22, 94, 101]. Indeed, the size of pores formed by both Bax and Bak depends on their membrane density [106, 107], in support of a pleomorphic set of pore sizes dependent on the number of protein subunits incorporated, and in which the building block is a protein dimer [108].

9. Toroidal lipidic pores

Underlying the existence of pores generated by a structural combination of protein subunits and lipid molecules, where the lipids have a non-lamellar arrangement, is the fact that lipids alone are capable of forming pores using a similar rearrangement without any proteins being involved (Figure 1d). This fact has been discussed in detail elsewhere [64, 66] and its relationship to pore formation

by proteins has been noted [7]. The transition of an electrical charge through membranes causes a reorientation of the lipids to generate a ring-shaped non-lamellar arrangement which, famously, allows the electroporation of DNA into cells [67]. The insertion of a permanent charge into the membrane – such as that provided by the charged amino acid sidechains of pore-forming proteins – can be expected to have a similar effect [16]. The structure of toroidal lipidic pores is characterised by a positive lipid curvature out (or rather, through) the plane of the membrane and a negative lipid curvature in the plane of the membrane (Figure 1d). Why should such structures be stable given that they are made simply out of lipids? Because the line tension of the membrane is reduced by the curvature of the lipids towards the hydrophobic core [7, 65]. Pore-forming proteins which generate hybrid protein-lipid pores piggyback on this basic property of biological bilayer membranes.

10. *Bend and snap*

The role of lipids in pore-forming structures generated by pore-forming proteins appears to confer a set of advantages. One is economy – it is no longer necessary to build a complete ring of subunits into a wall that defines a pore; rather, a limited number of subunits can harness the biophysical properties of phospholipids and the bilayer membranes they form to enable pore formation by intervention rather than direct construction. Another is flexibility – by avoiding the rigidity of a wall built brick-by-brick (subunit-by-subunit) the pore-forming mechanism builds in versatility, whether in terms of the capacity to form different sizes of pore in different contexts (e.g. pH [39, 43], protein concentration [14, 38, 42]) or in terms of a single pore, once formed, being deformable – as may be needed for apicomplexan migration through pores triggered by their perforin-like proteins [109], and seems likely to apply with direct travel of *Listeria* from cell to cell [43, 68]. The key achievement, however, is the opening of a channel through a single bilayer by the induction of a semi-toroidal, non-lamellar arrangement of lipids in-plane [7]. Membrane fusion proteins also direct the formation of highly curved lipidic structures, but these are the result of a bending of the bilayer out of plane to form a high-energy transition state, and the snapping together of the two membranes

as the transition state resolves [110, 111]. The opposite phenomenon, membrane fission, is brought about via the same intermediates as fusion, with however a quite different mechanism at the protein level based on, e.g., BAR domain proteins and dynamin [112, 113]. Whereas fusion is based on a mechanism pulling the membranes towards each other to form a pinched intermediate that easily resolves as the bilayers become confluent, fission begins with a pinching process as opposing regions of two membranes are pushed or drawn together (Figure 5a and d). Nevertheless, in both membrane fusion and fission the formation of a metastable intermediate structure involves a highly positively *and* negatively out-of-plane curved bilayer (Figure 5), whereas in pore formation we find an inherently stable non-lamellar structure in the plane of the bilayer with positive curvature through the membrane and negative curvature around the pore edge (Figures 1b, 2a, 2d and 4b). As discussed already, the stability of pore-forming toroids and semi-toroids is enabled by a reduction in line tension [7, 65]; the instability of the pinched-out structures formed during membrane fusion and fission most likely derives from charge repulsion effects and the much lower energy of the system if the membranes break open and fuse, or break up and separate (Figure 5). *En route*, fusion pores form a toroidal structure out of a highly bent bilayer rather than the highly curved transbilayer repacking of lipids; the resolution of fusion pores is a simple matter of relaxation as the membrane components redistribute themselves in-plane.

The critical steps in membrane fusion are the close approach of two membranes, leading to dehydration of their surfaces, the formation of a hemifusion intermediate and then of a fusion pore which relaxes as the two bilayers become fully confluent (Figure 5). Fusion proteins, like pore-forming proteins, harness basic characteristics of membrane bilayer biophysics to have their effects. This can perhaps be seen most simply in the case of the contribution to vesicle fusion provided by the proteolipid V_0 ring of vacuolar ATPases [114, 115]. Here, SNARE-mediated attachment of a vacuolar membrane to its target was shown to result in the apposition of two rings of V_0 subunits whose calmodulin-mediated disassembly is thought to enable lipid mixing leading to the actual

achievement of fusion [114, 115]. As V_0 subunits part lipids further infiltrate to plug the spaces between them, leading to the formation of a highly-curved between-bilayer fusion pore with a proteolipidic toroidal structure (Figure 5). This model remains the subject of research and discussion [116] – and other data indicate that only one of the fusing membranes requires a V_0 complex, while still seeing a fundamental role for this proteolipidic structure in the generation of membrane fusion based on the manipulation of basic biophysical properties of lipids [117]. In any case, this specific example highlights the fact that all fusion proteins need to do is coordinate the close apposition of two membranes and enable the resolution of the resulting junctional pore by in-plane relaxation. This last step is clearly critical – in fact the hemi-fusion state has some stability and needs reactivating for resolution to a fusion pore [110]. Proteins manipulate the basic physical properties of membranes in order to bring about membrane fusion – just as they do to bring about pore formation. Indeed a hemifusion stalk will form simply through the mutual energetic destabilisation of two closely-placed bilayer surfaces facilitated by dehydration by the effects of polyethyleneglycol [9, 118, 119].

The example of the V-ATPase V_0-ring is a rather special cellular case in which a non-professional fusion protein has been co-opted to a new role. Several examples of professional fusion proteins have been well characterised and described – for example viral fusion proteins from several lineages of viruses, and cellular fusion proteins [9, 110, 120, 121]. Traditionally, fusion proteins have been split into different families [120]: three classes of viral fusion proteins [9, 110], the intracellular SNAREs [122], homotypic endoplasmic reticulum "atlastin" fusion proteins [123, 124] and mitofusins [125]. However, the structure of baculovirus gp64 in a post-fusion state has suggested that all viral fusion proteins may share a common evolutionary origin [121], while cell-cell fusion proteins as found in *C. elegans* turn out to be clear homologues of class II viral fusion proteins as found in viruses such as flaviviruses (e.g. Dengue, Tick-borne encephalitis) [126] (Figure 5c). While pore-forming proteins have a common objective, they achieve it in a variety of

related ways (oligomerisation, protein-lipid interface formation, refolding, transitions in secondary structure). Despite their diversity (which is itself a matter of debate) fusion proteins show more similarity in basic mechanism, while existing in an exotic variety of structural forms.

The general nature of fusion mechanisms is highlighted by the role of mitofusins in mitochondrial fusion [125]. Mitofusins are, like mitochondrial fission proteins, members of the dynamin superfamily; although their precise mechanism is unclear, it is thought that both membranes approaching fusion contain a mitofusin and that their interaction leads to a pulling together of the membranes similar to the mechanisms of vesicle fusion driven by SNARE proteins [125]. That being said, it remains possible that a dynamin-like helical arrangement of the proteins is involved [125]; and yet, the actual mechanism should be similar to other fusion processes, whether carried out by professional viral fusion proteins or part-time operatives like the V_0 ring.

11. Starting in the same place

A final question should be posed. Given that they engage the same terrain and have to negotiate the same geography, are pore-forming and fusion proteins related to one another? Do at least some of them share a common evolutionary lineage? Are they homologous? This is a deep question, and it is a hard one to answer – though the solution of increasing numbers of structures of each class (importantly, in different states such as before target engagement, and after pore-formation or membrane fusion, as in the case of baculovirus gp64 [121]) will help to answer it as will our developing understanding of the ways in which structures can be used directly in phylogenetic analyses [127-131]. However, there do seem some superficial similarities between pore-forming and fusion proteins, as depicted in Figure 6, and alignment and calculation of root mean square differences in backbone equivalences enables the construction of a phylogenetic tree (Figure 7; see Table 1 for RMSD deviation and number of equivalent residues for pore-forming lysenin with

respect to each protein depicted). In particular, the pore-forming modules of lysenin and proaerolysin, pore-forming proteins which are known to be homologues of one another [15, 132], display some similarity to structures found in the Class III viral fusion proteins vesicular stomatitis virus glycoprotein (VSV-G) [133] and baculovirus gp64 [121]. In turn, VSV-G and gp64 might share with the influenza haemagglutinin [134] a common, long α-helix which is reminiscent of the structure of the *E. coli* cytolysin A (ClyA, see above) in its pore-forming state [29]. It is worth noting that for this comparison only acidic conditions, post-fusion state crystal structures of VSV-G, gp64 and haemagglutinin were used, alongside the pore-forming conformation of ClyA. The pore-forming structures of lysenin and aerolysin will in turn prove interesting comparison partners with the possibly equivalent regions on VSV-G and gp64 since these are not regions undergoing any substantial conformational changes during fusion, but during pore formation by lysenin and aerolysin they will. For example, is greater homology found between lysenin and aerolysin pore-forming modules and VSV-G and gp64 after pore formation has occurred and only the remnant core of the pore-forming domain remains, than with the soluble states of each protein shown in Figure 6?

How might structural phylogeny be robustly inferred? The work to date on viruses [127, 129, 131] and on pore-forming proteins [7, 36, 128, 130, 132] gives us some pointers. For example, do the phylogenetic trees derived from such comparisons follow kingdom relationships? What happens to the structural comparisons when structures not thought to be related are included in the comparisons? And, a reciprocal point, how do the numbers of equivalent residues with root-mean-square deviations of a given size (say, 2Å or less) compare within groups of proteins known to be related (e.g. pore-forming proteins of the lysenin/aerolysin class) and between such proteins and viral fusion proteins? The comparison shown in Figure 7 and Table 1 at first sight suggests that rooting of phylogenetic comparisons in subgroups of known pore-forming relatives and known fusion protein relatives could give some confidence that the subgroups are themselves related homologues. For sufficient numbers of cases, plots of root-mean-square deviation against numbers

of equivalent residues identified could also be informative – as also consideration of what criterion indicates “significant” homology. It has already been shown that subdomains of viral fusion proteins may be differentially conserved and indicate a single root for all such polypeptides [121] – and this thinking should also be applied to the comparison of pore-forming and fusion proteins. In the end perhaps the strongest arguments will remain that there do seem to be multiple protein architectures capable of triggering fusion or bringing about pore formation, and yet similarities seem to exist between the two groups of proteins. Those similarities may turn out to be trivial, or convergent, but the possibility that they betray divergent evolution from a common ancestor deserves further study and should not be discounted at this stage. Why should, or might, such relationships exist? Pore-forming and fusion proteins face a related set of challenges centred on the remodelling of membranes; it would not be so surprising in the end if it turned out that the capacity of certain protein structures to engage with membranes in ways which destabilise and remodel lipid bilayers had been strongly conserved. Indeed, we know from the paradigm case of the MACPF/CDC proteins that exactly such a selective pressure exists [71, 135].

Figures

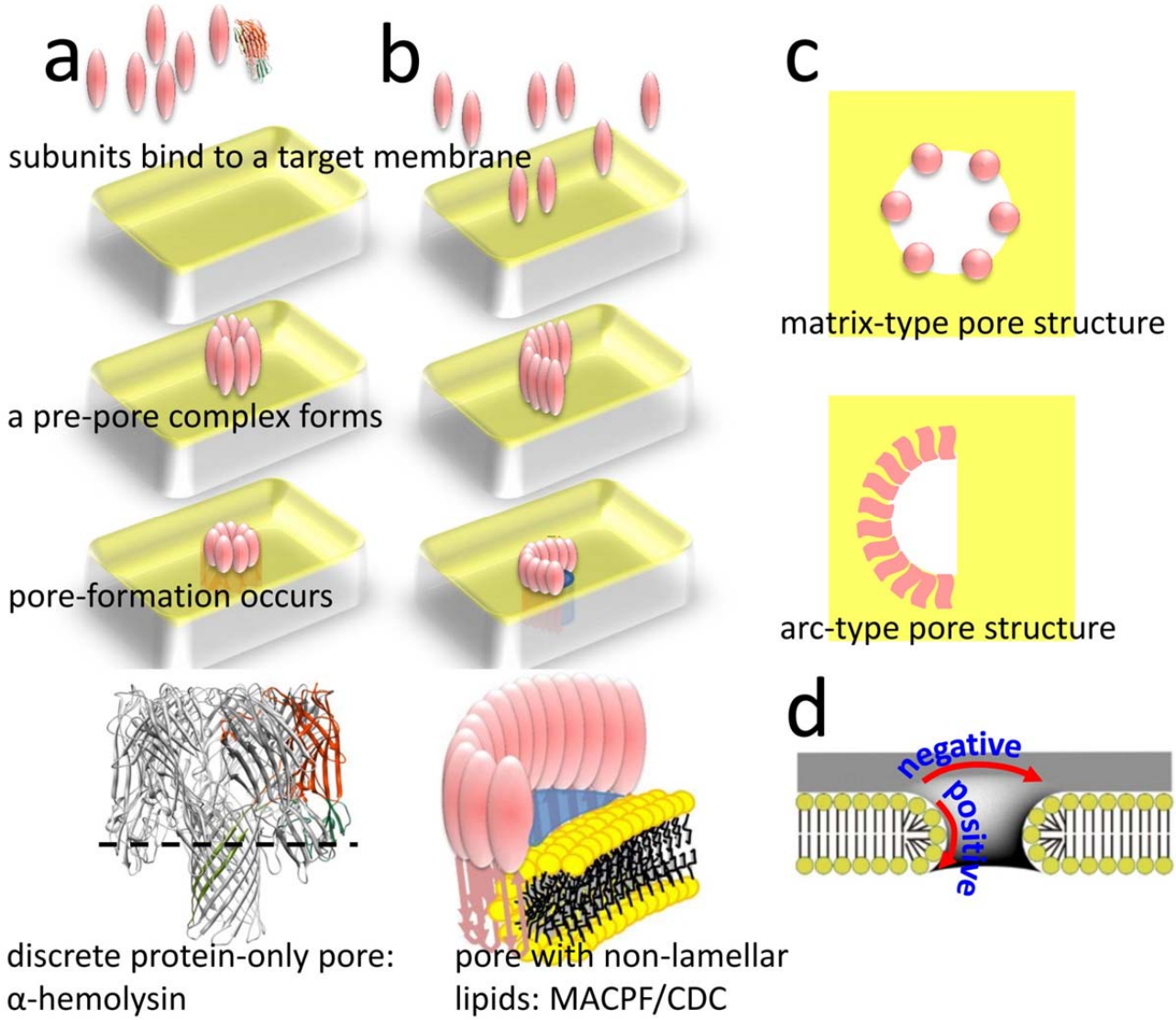

Figure 1

Schematic diagrams of pore formation.

a) Pore formation by proteins such as *Staphylococcus aureus* α-hemolysin, in which (in this case) seven subunits come together in a pre-pore structure before transitioning to a membrane inserted pore. The bottom image shows a ribbon format representation of the α-hemolysin pore crystal structure [30], while one of the schematic monomers in the top image is superposed by a representative monomer from this protein family, the LukF

bicomponent endotoxin [61]. In the pore structure the dashed line indicates the upper surface of the lipid bilayer, while one subunit is coloured against the grey rendering of the other six.

b) Pore formation by membrane attack complex/perforin-cholesterol dependent cytolysin (MACPF/CDC) proteins. This scheme is also relevant to the Bax/colicin family of proteins [22, 101], though see Figure 3b for more details. Although complete rings of subunits do form pores, the MACPF/CDC proteins distinctively can also act using arcs of subunits interfacing with a non-lamellar, semi-toroidal lipidic edge, as shown in the expanded cartoon provided as the fourth image in the sequence [7, 14, 16, 36, 41, 42, 45]. Like α-hemolysin, the MACPF/CDCs go through a pre-pore to pore transition; unlike α-hemolysin this can occur at any stage [7, 39, 40] beyond (it is estimated) five subunits [38].

c) Schematics of matrix-type and arc-type pore-forming structures in which the lipid (yellow) forms a proportion of the channel perimeter as organised by the protein (pink).

d) Cartoon of pore formation within a lipid bilayer by a toroidal rearrangement of the lipids alone [7, 64-66], cut through to show the positive curvature through the lipid plane and the negative curvature of lipids around the pore perimeter. Reprinted with modification from [7] by permission of Elsevier.

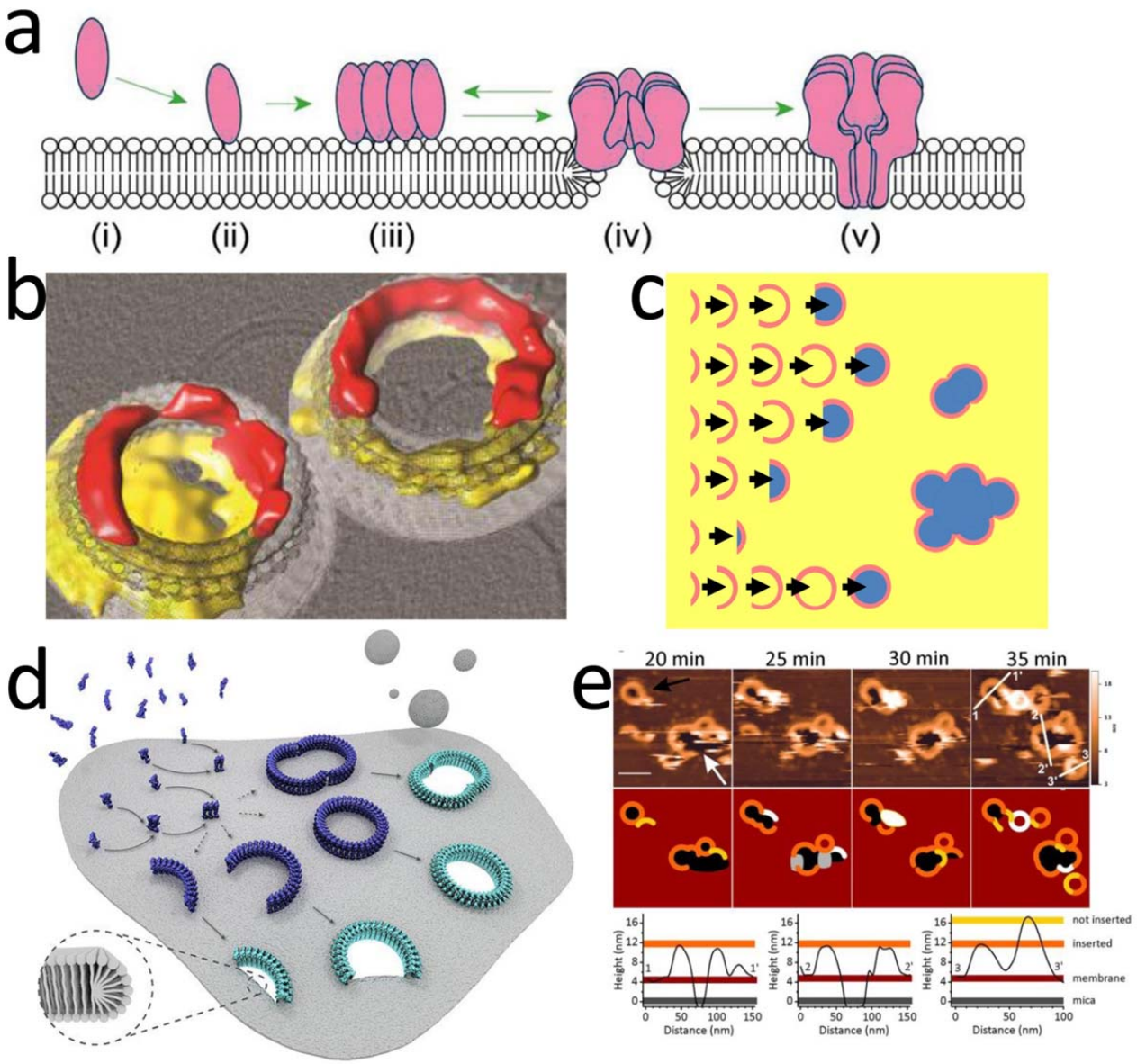

Figure 2

Lipids in pore structures formed by β-sheeted pore-forming proteins

a) A toroidal arrangement of lipids may facilitate the membrane insertion of α-hemolysin pre-pores, in the sequence numbered from (i) to (v) [24]. Figure modified from [24] and reproduced with permission, © the authors.

b) Pre-pore (*left*) and pore (*right*) structures formed by arcs of the MACPF/CDC pneumolysin, on the background of a section through one of the cryo-electron tomograms from which these sub-tomogram averages were computed. Lipid is coloured yellow, protein red [40].

c) A schematic model for the kinetically-determined assembly of MACPF/CDC proteins into pores using diverse sizes of oligomer. Once the local supply of free subunits is exhausted, pre-pore to pore transition occurs. This model has been described in a number of reviews [14, 16, 42, 71] and quantitatively confirmed [38]. Also relevant is data shown in panel **e** from [39].

d) Schematic of membrane binding, oligomerisation and pore formation by the MACPF/CDC suilysin, from [38]. Monomers bind (left), oligomerise into arcs and rings and then form pores. Kinetically-trapped arcs are as capable of pore formation as full rings, and can then associate together to create double-arc pores. This image is © Leung et al. (2014).

e) Time sequence of atomic force microscopy images of the MACPF/CDC listeriolysin forming pores on a supported membrane, from [39]. Arcs are seen to generate pores and also to associate together into "patchwork" structures possessing much greater conductance capacity. The upper row shows actual AFM images, the middle row schematics inferred directly from them, the bottom row height profiles along the lines indicated in the 35 minute image. The growth and fusion of new structures with an already-formed oligomeric ring is seen (black arrow in 20 minute image) as well as formation of a patchwork pore (white arrow). This image is © Podobnik et al. (2015).

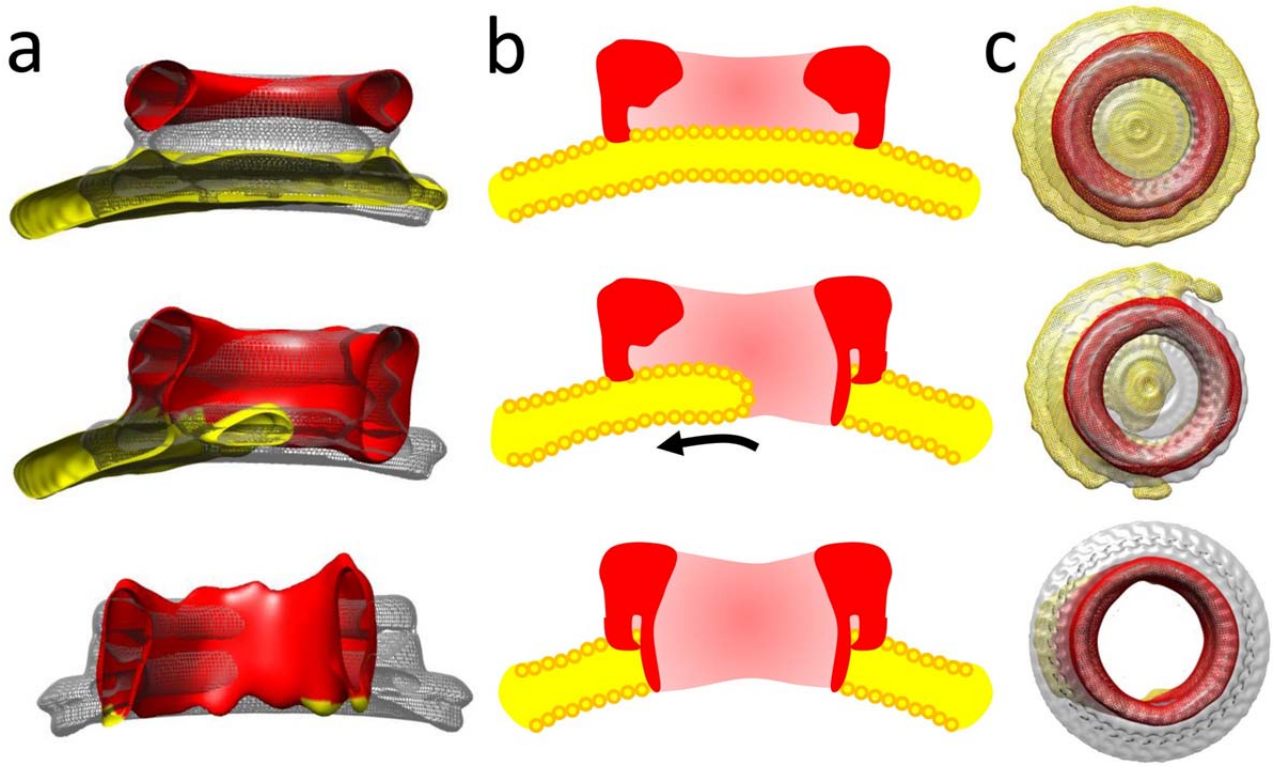

Figure 3

Pore formation by the gradual insertion of MACPF/CDC pores.

a) Side views of pneumolysin oligomers within lipid bilayers as resolved using sub-tomogram averaging: top, pre-pore; middle, partial pore or partially-inserted oligomer; bottom, pore.

b) Cartoons indicating the inferred state of the membrane and protein in each case shown in **a**. The rolling insertion of the oligomer, starting from the right hand edge as viewed here and in **a** results in the flow of lipids back into the bilayer (arrow).

c) Top views of the same sub-tomogram averages shown in **a**.

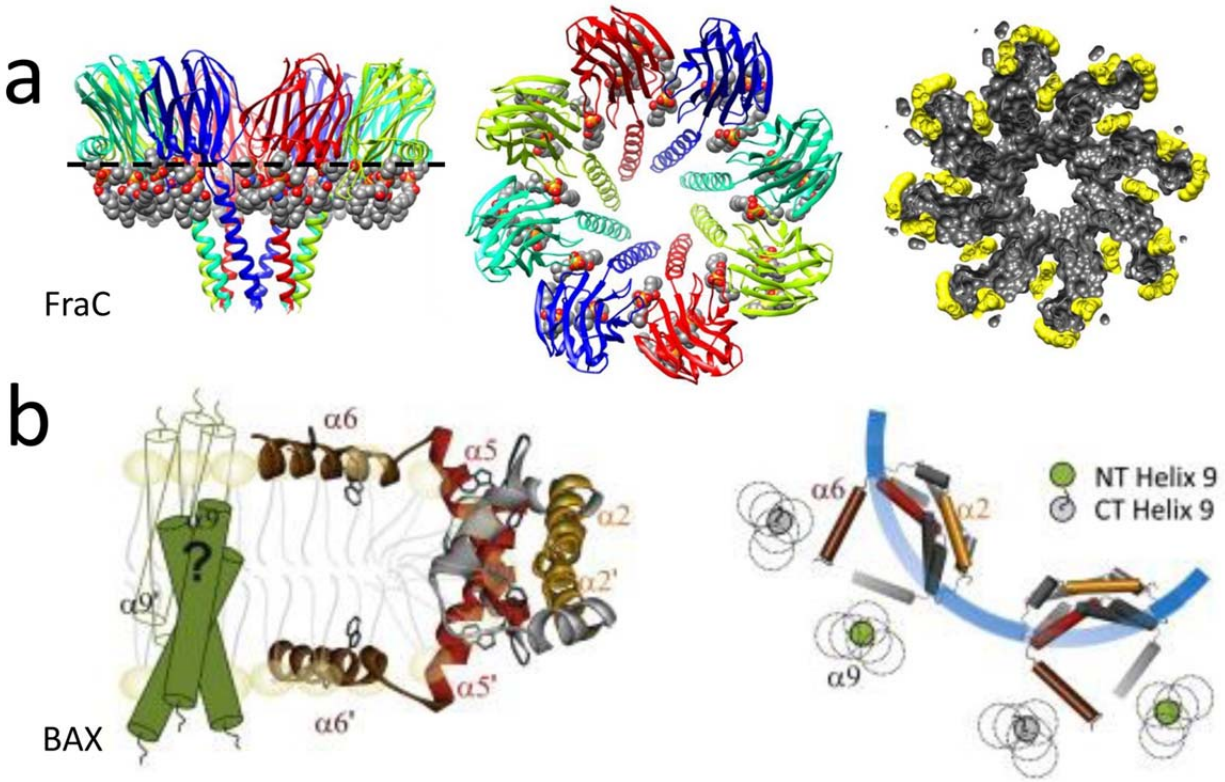

Figure 4

Lipids in pore structures formed by α-helical pore-forming proteins.

a) Lipids play a clear structural role in the actinoporins; they have been resolved forming part of the FraC pore structure [93]. Three views of the pore are shown – on the left from the side and in the centre from above, with the subunits in ribbon format coloured disctinctively and the three lipids per subunit involved in the pore structure shown in a space-filling coloured by element. In the left hand image the dashed black line indicates the upper edge of the membrane, as also shown by the lipid headgroup positions. In the right hand image, a top view is shown with the FraC subunits displayed in a surface-rendered format in grey, cut across just below the upper membrane surface with the lipids shown as space-filling models in yellow. It is clear here that the lipids provide a significant proportion of the channel perimeter.

b) Images taken from Bleicken and colleagues [22], reproduced with permission and © Elsevier, Inc. of a proposed model for the Bax pore. Double electron-electron resonance

measurements in liposomes and isolated mitochondria enabled the measurement of pairwise distances leading to this model for the remodelling of lipid bilayers into a non-lamellar, semi-toroidal arrangement by an activated dimer of Bax. The Bax dimer has also been resolved crystallographically [105] and a similar model has been proposed by Westphal and colleagues [101]. On the left is shown a view in profile, with a single Bax dimer; on the right a view from above in which a pair of Bax dimers trigger lipid-based pore formation. On the right, the blue arc marks the proposed edge of the pore, and the specific labelling of helix 9 underscores the antiparallel dimerization undergone by Bax [105], as also apparent from the left hand image.

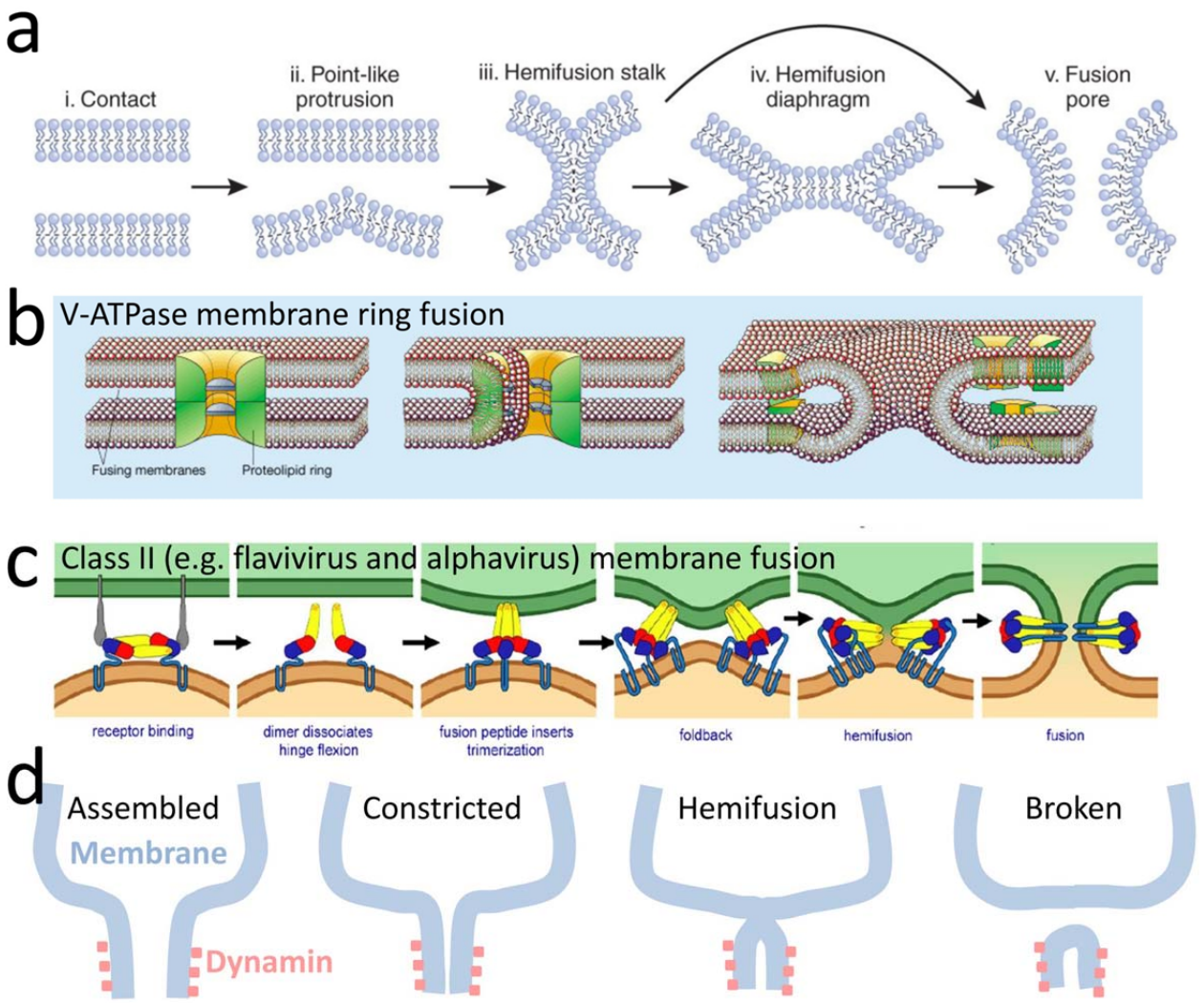

Figure 5

Mechanisms of membrane fusion and fission.

(a) Sequential steps in membrane fusion, modified from [6] and reproduced with permission, © Nature Publishing Group. Two apposed membranes (i) approach one another whereupon (ii) a point-like membrane protrusion arises from a minimisation of the hydration repulsion between the two membranes' proximal leaflets. Further dehydration results in formation of the hemifusion stalk (iii) followed by stalk expansion (iv) to yield a hemifusion diaphragm or nascent fusion pore which opens up to generate the fusion pore proper (v) which relaxes as the two membranes become fully confluent. Alternatively, the hemifusion stalk can transition directly to a fusion pore. See also [9, 110, 122, 136] and for a molecular dynamic simulation [137].

(b) Membrane fusion as observed in yeast vacuoles, mediated by the proteolipidic V_0 ring of the vacuolar ATPase [114, 115]. Apposition of two V_0 rings [115] (or action of a single ring [117]), in a mechanism activated by calmodulin, leads to the disassembly of the octameric V_0 and membrane fusion, in a variant of the scheme shown in panel **a** whereby the V_0 facilitates or scaffolds fusion pore formation. Figure modified from [114] with permission and © Macmillan Publishing Ltd.

(c) Membrane fusion as carried out by Class II viral fusion proteins, such as dengue virus. This figure is modified from [9] and [138] with permission and is © the author, Stephen C. Harrison.

(d) Membrane fission as forced by dynamin; a similar mechanism applies also with BAR domain proteins, whereby a neck structure is constricted and breaks apart [112, 113]. The figure is drawn after Figure 3 of [113].

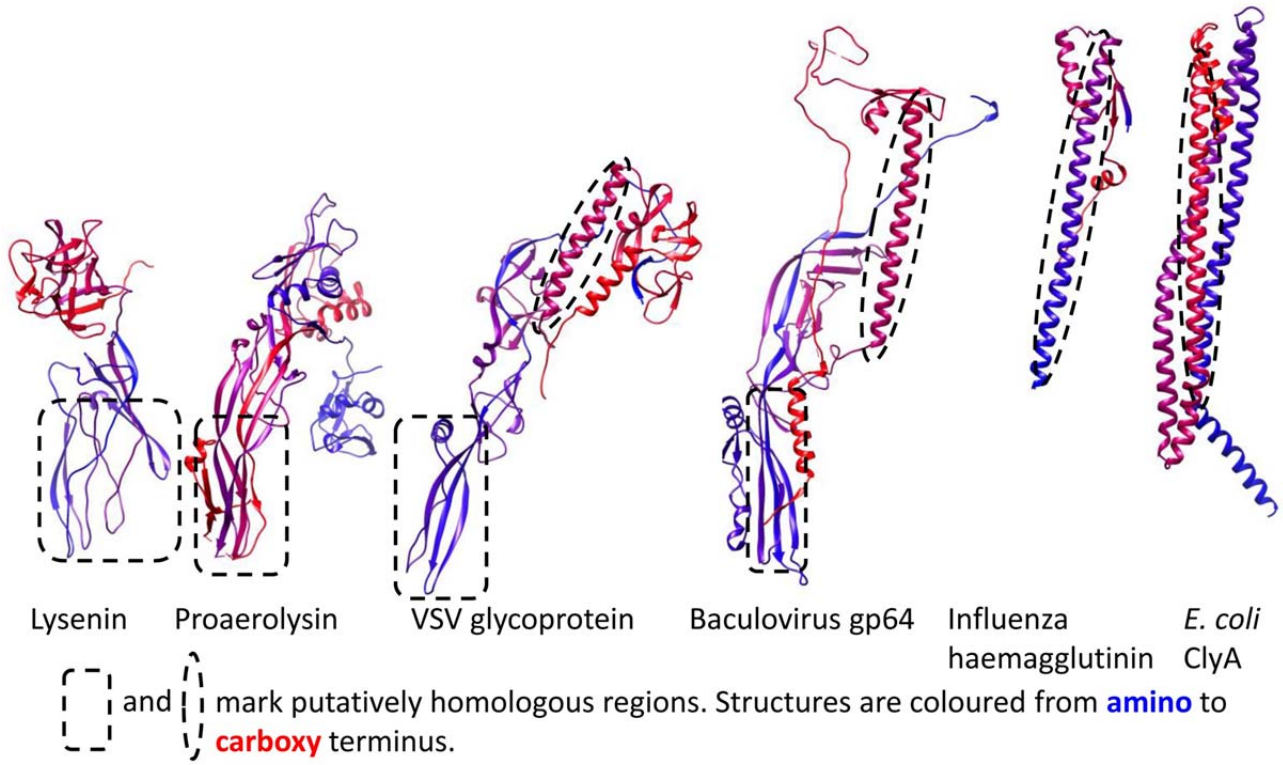

Figure 6

Side-by-side comparison of some pore-forming and fusion proteins.

Each protein is shown in ribbon format and coloured from blue at its amino-terminus to red at its carboxy-terminus. Possibly homologous regions are boxed or circled with dashed lines. The following structures were used: lysenin (PDB ID: 3ZXD); proaerolysin (PDB ID: 1PRE); VSV-G (PDB ID: 2CMZ); gp64 (PDB ID: 3DUZ); influenza haemagglutinin (PDB ID: 1HTM) and ClyA (PDB ID: 2WCD).

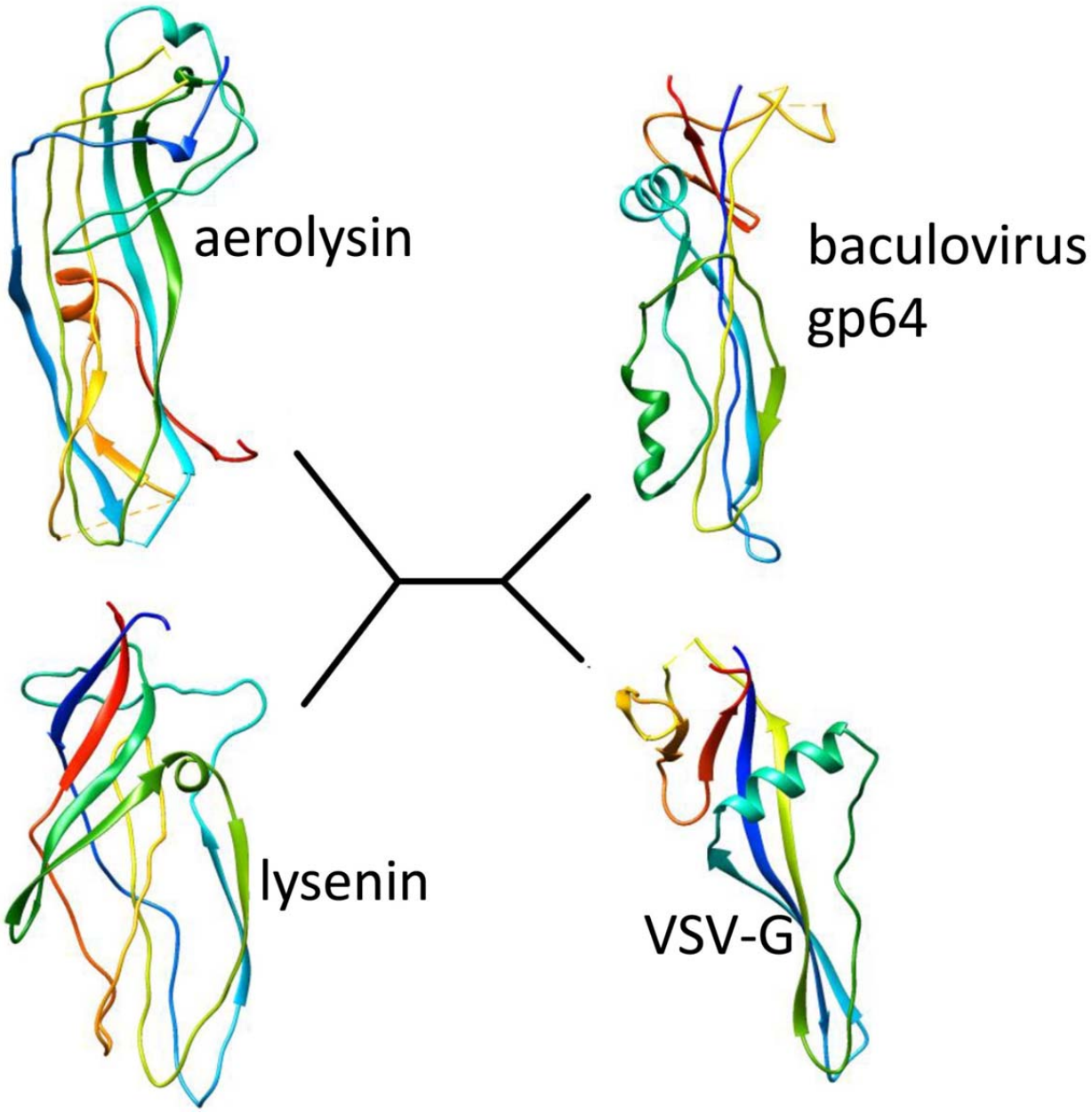

Figure 7

Phylogenetic analysis of putatively equivalent regions in pore-forming and fusion proteins.

The regions boxed in Figure 5 are shown coloured from blue at the amino terminus to red at the carboxy terminus, on a phylogenetic tree constructed via SHP [139] as described previously [140] and plotted using the programs FITCH and DRAWTREE as part of the PHYLIP package [141]. See Table 1 for RMSD values and numbers of equivalences.

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Table 1

2→ **1↓**	Lysenin	Aerolysin	Baculovirus gp64	VSV glycoprotein
Lysenin		**1.74 (47)**	**1.48 (28)**	**1.89 (28)**
Aerolysin	**1.89 (46)**		**1.78 (29)**	**1.76 (22)**
Baculovirus gp64	**1.61 (26)**	**2.03 (27)**		**1.79 (62)**
VSV glycoprotein	**1.70 (20)**	**1.92 (37)**	**1.75 (60)**	

A table of root mean square (RMS) deviations between structures aligned by pairwise structural homology and the number of structurally equivalent residues over which the deviation was calculated, all with reference to the lysenin pore-forming module. The alignments were made by pairwise fitting (**1** on **2**) of the equivalent regions of each protein using SHP [139] as previously reported [140].

References

[1] A.V. Collins, D.W. Brodie, R.J. Gilbert, A. Iaboni, R. Manso-Sancho, B. Walse, D.I. Stuart, P.A. van der Merwe, S.J. Davis, The interaction properties of costimulatory molecules revisited, Immunity, 17 (2002) 201-210.

[2] S.J. Davis, P.A. van der Merwe, Lck and the nature of the T cell receptor trigger, Trends Immunol, 32 (2011) 1-5.

[3] M. Schmick, P.I. Bastiaens, The interdependence of membrane shape and cellular signal processing, Cell, 156 (2014) 1132-1138.

[4] K. Simons, M.J. Gerl, Revitalizing membrane rafts: new tools and insights, Nat Rev Mol Cell Biol, 11 (2010) 688-699.

[5] J. Bigay, B. Antonny, Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity, Dev Cell, 23 (2012) 886-895.

[6] L.V. Chernomordik, M.M. Kozlov, Mechanics of membrane fusion, Nat Struct Mol Biol, 15 (2008) 675-683.

[7] R.J. Gilbert, M. Dalla Serra, C.J. Froelich, M.I. Wallace, G. Anderluh, Membrane pore formation at protein-lipid interfaces, Trends Biochem Sci, 39 (2014) 510-516.

[8] J. Rizo, T.C. Sudhof, The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices--guilty as charged?, Annu Rev Cell Dev Biol, 28 (2012) 279-308.

[9] S.C. Harrison, Viral membrane fusion, Virology, 479-480 (2015) 498-507.

[10] G. Anderluh, M. Dalla Serra, G. Viero, G. Guella, P. Macek, G. Menestrina, Pore formation by equinatoxin II, a eukaryotic protein toxin, occurs by induction of nonlamellar lipid structures, J Biol Chem, 278 (2003) 45216-45223.

[11] C.L. Bashford, G. Menestrina, P.A. Henkart, C.A. Pasternak, Cell damage by cytolysin. Spontaneous recovery and reversible inhibition by divalent cations, J Immunol, 141 (1988) 3965-3974.

[12] G. Menestrina, C.L. Bashford, C.A. Pasternak, Pore-forming toxins: experiments with S. aureus alpha-toxin, C. perfringens theta-toxin and E. coli haemolysin in lipid bilayers, liposomes and intact cells, Toxicon, 28 (1990) 477-491.

[13] C.A. Valcarcel, M. Dalla Serra, C. Potrich, I. Bernhart, M. Tejuca, D. Martinez, F. Pazos, M.E. Lanio, G. Menestrina, Effects of lipid composition on membrane permeabilization by sticholysin I and II, two cytolysins of the sea anemone Stichodactyla helianthus, Biophys J, 80 (2001) 2761-2774.

[14] R.J. Gilbert, Pore-forming toxins, Cell Mol Life Sci, 59 (2002) 832-844.

[15] M.W. Parker, J.T. Buckley, J.P. Postma, A.D. Tucker, K. Leonard, F. Pattus, D. Tsernoglou, Structure of the Aeromonas toxin proaerolysin in its water-soluble and membrane-channel states, Nature, 367 (1994) 292-295.

[16] R.J. Gilbert, Inactivation and activity of cholesterol-dependent cytolysins: what structural studies tell us, Structure, 13 (2005) 1097-1106.

[17] J. Rossjohn, G. Polekhina, S.C. Feil, C.J. Morton, R.K. Tweten, M.W. Parker, Structures of perfringolysin O suggest a pathway for activation of cholesterol-dependent cytolysins, J Mol Biol, 367 (2007) 1227-1236.

[18] A.S. Solovyova, M. Nollmann, T.J. Mitchell, O. Byron, The solution structure and oligomerization behavior of two bacterial toxins: pneumolysin and perfringolysin O, Biophys J, 87 (2004) 540-552.

[19] R.J. Gilbert, R.K. Heenan, P.A. Timmins, N.A. Gingles, T.J. Mitchell, A.J. Rowe, J. Rossjohn, M.W. Parker, P.W. Andrew, O. Byron, Studies on the structure and mechanism of a bacterial protein toxin by analytical ultracentrifugation and small-angle neutron scattering, J Mol Biol, 293 (1999) 1145-1160.

[20] R.J. Gilbert, J.L. Jimenez, S. Chen, I.J. Tickle, J. Rossjohn, M. Parker, P.W. Andrew, H.R. Saibil, Two structural transitions in membrane pore formation by pneumolysin, the pore-forming toxin of Streptococcus pneumoniae, Cell, 97 (1999) 647-655.

[21] M. Marchioretto, M. Podobnik, M. Dalla Serra, G. Anderluh, What planar lipid membranes tell us about the pore-forming activity of cholesterol-dependent cytolysins, Biophys Chem, 182 (2013) 64-70.

[22] S. Bleicken, G. Jeschke, C. Stegmueller, R. Salvador-Gallego, A.J. Garcia-Saez, E. Bordignon, Structural model of active Bax at the membrane, Mol Cell, 56 (2014) 496-505.

[23] P.E. Czabotar, G. Lessene, A. Strasser, J.M. Adams, Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy, Nat Rev Mol Cell Biol, 15 (2014) 49-63.

[24] D. Stoddart, M. Ayub, L. Hoefler, P. Raychaudhuri, J.W. Klingelhoefer, G. Maglia, A. Heron, H. Bayley, Functional truncated membrane pores, Proc Nat'l Acad Sci USA, 111 (2014) 2425-2430.

[25] G. Anderluh, J.H. Lakey, Disparate proteins use similar architectures to damage membranes, Trends Biochem Sci, 33 (2008) 482-490.

[26] M.W. Parker, S.C. Feil, Pore-forming protein toxins: from structure to function, Progr Biophys Mol Biol, 88 (2005) 91-142.

[27] O. Shatursky, A.P. Heuck, L.A. Shepard, J. Rossjohn, M.W. Parker, A.E. Johnson, R.K. Tweten, The mechanism of membrane insertion for a cholesterol-dependent cytolysin: a novel paradigm for pore-forming toxins, Cell, 99 (1999) 293-299.

[28] L.A. Shepard, A.P. Heuck, B.D. Hamman, J. Rossjohn, M.W. Parker, K.R. Ryan, A.E. Johnson, R.K. Tweten, Identification of a membrane-spanning domain of the thiol-activated pore-forming toxin Clostridium perfringens perfringolysin O: an alpha-helical to beta-sheet transition identified by fluorescence spectroscopy, Biochemistry, 37 (1998) 14563-14574.

[29] M. Mueller, U. Grauschopf, T. Maier, R. Glockshuber, N. Ban, The structure of a cytolytic alpha-helical toxin pore reveals its assembly mechanism, Nature, 459 (2009) 726-730.

[30] L. Song, M.R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, J.E. Gouaux, Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore, Science 274 (1996) 1859-1866.

[31] K. Yamashita, Y. Kawai, Y. Tanaka, N. Hirano, J. Kaneko, N. Tomita, M. Ohta, Y. Kamio, M. Yao, I. Tanaka, Crystal structure of the octameric pore of staphylococcal gamma-hemolysin reveals the beta-barrel pore formation mechanism by two components, Proc Nat'l Acad Sci USA, 108 (2011) 17314-17319.

[32] A. Badarau, H. Rouha, S. Malafa, D.T. Logan, M. Hakansson, L. Stulik, I. Dolezilkova, A. Teubenbacher, K. Gross, B. Maierhofer, S. Weber, M. Jagerhofer, D. Hoffman, E. Nagy, Structure-function analysis of heterodimer formation, oligomerization, and receptor binding of the Staphylococcus aureus bi-component toxin LukGH, J Biol Chem, 290 (2015) 142-156.

[33] G.M. Coates, C.L. Bashford, O.S. Smart, Using HOLE to predict the effects of PEG's on the conductance of alpha-toxin, Biochem Soc Trans, 26 (1998) S193.

[34] J. Jiang, B.L. Pentelute, R.J. Collier, Z.H. Zhou, Atomic structure of anthrax protective antigen pore elucidates toxin translocation, Nature, 521 (2015) 545-549.

[35] C. Petosa, R.J. Collier, K.R. Klimpel, S.H. Leppla, R.C. Liddington, Crystal structure of the anthrax toxin protective antigen, Nature, 385 (1997) 833-838.

[36] R.J. Gilbert, M. Mikelj, M. Dalla Serra, C.J. Froelich, G. Anderluh, Effects of MACPF/CDC proteins on lipid membranes, Cell Mol Life Sci, 70 (2013) 2083-2098.

[37] S.J. Tilley, E.V. Orlova, R.J. Gilbert, P.W. Andrew, H.R. Saibil, Structural basis of pore formation by the bacterial toxin pneumolysin, Cell, 121 (2005) 247-256.

[38] C. Leung, N.V. Dudkina, N. Lukoyanova, A.W. Hodel, I. Farabella, A.P. Pandurangan, N. Jahan, M. Pires Damaso, D. Osmanovic, C.F. Reboul, M.A. Dunstone, P.W. Andrew, R. Lonnen, M. Topf, H.R. Saibil, B.W. Hoogenboom, Stepwise visualization of membrane pore formation by suilysin, a bacterial cholesterol-dependent cytolysin, eLife, 3 (2014) e04274.

[39] M. Podobnik, M. Marchioretto, M. Zanetti, A. Bavdek, M. Kisovec, M.M. Cajnko, L. Lunelli, M. Dalla Serra, G. Anderluh, Plasticity of listeriolysin O pores and its regulation by pH and a unique histidine, Sci Rep, 5 (2015) 9623.

[40] A.F. Sonnen, J. Plitzko, R.J.C. Gilbert, Incomplete pneumolysin oligomers form membrane pores, Open Biol, 4 (2014) 140044.

[41] S. Bhakdi, J. Tranum-Jensen, A. Sziegoleit, Mechanism of membrane damage by streptolysin-O, Infect Immun, 47 (1985) 52-60.

[42] R.J. Gilbert, Cholesterol-dependent cytolysins, Adv Exp Med Biol, 677 (2010) 56-66.

[43] C.L. Birmingham, V. Canadien, N.A. Kaniuk, B.E. Steinberg, D.E. Higgins, J.H. Brumell, Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles, Nature, 451 (2008) 350-354.

[44] S. Metkar, M. Marchioretto, V. Antonini, L. Lunelli, B. Wang, R.J.C. Gilbert, G. Anderluh, R. Roth, M. Pooga, J. Pardo, J.E. Heuser, M. Dalla Serra, C.J. Froelich, Perforin oligomers form arcs in cellular membranes: a locus for intracellular delivery of granzymes, Cell Death Dis, 22 (2015) 78-85.

[45] M. Palmer, R. Harris, C. Freytag, M. Kehoe, J. Tranum-Jensen, S. Bhakdi, Assembly mechanism of the oligomeric streptolysin O pore: the early membrane lesion is lined by a free edge of the lipid membrane and is extended gradually during oligomerization, EMBO J, 17 (1998) 1598-1605.

[46] T. Praper, A.F. Sonnen, G. Viero, A. Kladnik, C.J. Froelich, G. Anderluh, M. Dalla Serra, R.J. Gilbert, Human perforin employs different avenues to damage membranes, J Biol Chem, 286 (2011) 2946-2955.

[47] J. Thiery, D. Keefe, S. Boulant, E. Boucrot, M. Walch, D. Martinvalet, I.S. Goping, R.C. Bleackley, T. Kirchhausen, J. Lieberman, Perforin pores in the endosomal membrane trigger the release of endocytosed granzyme B into the cytosol of target cells, Nat Immunol, 12 (2011) 770-777.

[48] Z.O. Shenkarev, S.V. Balandin, K.I. Trunov, A.S. Paramonov, S.V. Sukhanov, L.I. Barsukov, A.S. Arseniev, T.V. Ovchinnikova, Molecular mechanism of action of beta-hairpin antimicrobial peptide arenicin: oligomeric structure in dodecylphosphocholine micelles and pore formation in planar lipid bilayers, Biochemistry, 50 (2011) 6255-6265.

[49] L. Prieto, Y. He, T. Lazaridis, Protein arcs may form stable pores in lipid membranes, Biophy J, 106 (2014) 154-161.

[50] H. Jang, L. Connelly, F.T. Arce, S. Ramachandran, B.L. Kagan, R. Lal, R. Nussinov, Mechanisms for the Insertion of Toxic, Fibril-like beta-Amyloid Oligomers into the Membrane, J Chem Theor Comput, 9 (2013) 822-833.

[51] M. Mihajlovic, T. Lazaridis, Antimicrobial peptides in toroidal and cylindrical pores, Biochim Biophys Acta, 1798 (2010) 1485-1493.

[52] K.A. Brogden, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?, Nat Rev Microbiol, 3 (2005) 238-250.

[53] R. Capone, M. Mustata, H. Jang, F.T. Arce, R. Nussinov, R. Lal, Antimicrobial protegrin-1 forms ion channels: molecular dynamic simulation, atomic force microscopy, and electrical conductance studies, Biophys J, 98 (2010) 2644-2652.

[54] H. Jang, J. Zheng, R. Lal, R. Nussinov, New structures help the modeling of toxic amyloidbeta ion channels, Trends Biochem Sci, 33 (2008) 91-100.

[55] H. Jang, F.T. Arce, S. Ramachandran, R. Capone, R. Lal, R. Nussinov, beta-Barrel topology of Alzheimer's beta-amyloid ion channels, J Mol Biol, 404 (2010) 917-934.

[56] B. Walker, O. Braha, S. Cheley, H. Bayley, An intermediate in the assembly of a pore-forming protein trapped with a genetically-engineered switch, Chem Biol, 2 (1995) 99-105.

[57] T. Kawate, E. Gouaux, Arresting and releasing Staphylococcal alpha-hemolysin at intermediate stages of pore formation by engineered disulfide bonds, Protein Sci, 12 (2003) 997-1006.

[58] A.E. Aleshin, I.U. Schraufstatter, B. Stec, L.A. Bankston, R.C. Liddington, R.G. Discipio, Structure of Complement C6 suggests a mechanism for initiation and unidirectional, sequential assembly of the Membrane Attack Complex (MAC), J Biol Chem, 287 (2012) 10210–10222

[59] L.L. Lovelace, C.L. Cooper, J.M. Sodetz, L. Lebioda, Structure of human C8 protein provides mechanistic insight into membrane pore formation by complement, J Biol Chem, 286 (2011) 17585-17592.

[60] A.F. Sonnen, P. Henneke, Structural biology of the membrane attack complex, Sub-cell Biochem, 80 (2014) 83-116.

[61] R. Olson, H. Nariya, K. Yokota, Y. Kamio, E. Gouaux, Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel, Nat Struct Biol, 6 (1999) 134-140.

[62] O.S. Smart, G.M. Coates, M.S. Sansom, G.M. Alder, C.L. Bashford, Structure-based prediction of the conductance properties of ion channels, Faraday Discuss, 111 (1998) 185-199.

[63] J.R. Thompson, B. Cronin, H. Bayley, M.I. Wallace, Rapid assembly of a multimeric membrane protein pore, Biophys J, 101 (2011) 2679-2683.

[64] E. Karatekin, O. Sandre, H. Guitouni, N. Borghi, P.H. Puech, F. Brochard-Wyart, Cascades of transient pores in giant vesicles: line tension and transport, Biophys J, 84 (2003) 1734-1749.

[65] S. May, A molecular model for the line tension of lipid membranes, Eur Phys J E, 3 (2000) 37-44.

[66] J. Teissie, M. Golzio, M.P. Rols, Mechanisms of cell membrane electropermeabilization: a minireview of our present (lack of ?) knowledge, Biochim Biophys Acta, 1724 (2005) 270-280.

[67] J.C. Weaver, Molecular basis for cell membrane electroporation, Ann N Y Acad Sci, 720 (1994) 141-152.

[68] M.A. Czuczman, R. Fattouh, J.M. van Rijn, V. Canadien, S. Osborne, A.M. Muise, V.K. Kuchroo, D.E. Higgins, J.H. Brumell, Listeria monocytogenes exploits efferocytosis to promote cell-to-cell spread, Nature, 509 (2014) 230-234.

[69] T. Borsos, R.R. Dourmashkin, J.H. Humphrey, Lesions in Erythrocyte Membranes Caused by Immune Haemolysis, Nature, 202 (1964) 251-252.

[70] J. Tschopp, Ultrastructure of the membrane attack complex of complement. Heterogeneity of the complex caused by different degree of C9 polymerization, J Biol Chem, 259 (1984) 7857-7863.

[71] R.J.C. Gilbert, Perforins, in: A. Delcour (Ed.) Electrophysiology of Unconventional Channels and Pores, Springer, Dordrecht/NL, 2015.

[72] M. Palmer, A. Valeva, M. Kehoe, S. Bhakdi, Kinetics of streptolysin O self-assembly, Eur J Biochem, 231 (1995) 388-395.

[73] S. Bhakdi, J. Tranum-Jensen, Complement lysis: a hole is a hole, Immunology today, 12 (1991) 318-320; discussion 321.

[74] E.M. Hotze, R.K. Tweten, Membrane assembly of the cholesterol-dependent cytolysin pore complex, Biochim Biophys Acta, 1818 (2012) 1028-1038.

[75] M.A. Dunstone, R.K. Tweten, Packing a punch: the mechanism of pore formation by cholesterol dependent cytolysins and membrane attack complex/perforin-like proteins, Curr Op Struct Biol, 22 (2012) 342-349.

[76] N. Lukoyanova, H.R. Saibil, Friend or foe: the same fold for attack and defense, Trends Immunol, 29 (2008) 51-53.

[77] R.K. Tweten, E.M. Hotze, K.R. Wade, The Unique Molecular Choreography of Giant Pore Formation by the Cholesterol-Dependent Cytolysins of Gram-Positive Bacteria, Annu Rev Microbiol, 69 (2015) 323-340.

[78] E.R. Podack, G. Dennert, Assembly of two types of tubules with putative cytolytic function by cloned natural killer cells, Nature, 302 (1983) 442-445.

[79] J.D. Young, H. Hengartner, E.R. Podack, Z.A. Cohn, Purification and characterization of a cytolytic pore-forming protein from granules of cloned lymphocytes with natural killer activity, Cell, 44 (1986) 849-859.

[80] Y.E. Korchev, C.L. Bashford, C. Pederzolli, C.A. Pasternak, P.J. Morgan, P.W. Andrew, T.J. Mitchell, A conserved tryptophan in pneumolysin is a determinant of the characteristics of channels formed by pneumolysin in cells and planar lipid bilayers, Biochem J, 329 (1998) 571-577.

[81] S.S. Metkar, B. Wang, E. Catalan, G. Anderluh, R.J. Gilbert, J. Pardo, C.J. Froelich, Perforin rapidly induces plasma membrane phospholipid flip-flop, PLoS One, 6 (2011) e24286.

[82] A.A. Sobko, E.A. Kotova, Y.N. Antonenko, S.D. Zakharov, W.A. Cramer, Lipid dependence of the channel properties of a colicin E1-lipid toroidal pore, J Biol Chem, 281 (2006) 14408-14416.

[83] A. Pokorny, P.F. Almeida, Kinetics of dye efflux and lipid flip-flop induced by delta-lysin in phosphatidylcholine vesicles and the mechanism of graded release by amphipathic, alpha-helical peptides, Biochemistry, 43 (2004) 8846-8857.

[84] D.M. Czajkowsky, E.M. Hotze, Z. Shao, R.K. Tweten, Vertical collapse of a cytolysin prepore moves its transmembrane beta-hairpins to the membrane, EMBO J, 23 (2004) 3206-3215.

[85] P. Amiguet, J. Brunner, J. Tschopp, The membrane attack complex of complement: lipid insertion of tubular and nontubular polymerized C9, Biochemistry, 24 (1985) 7328-7334.

[86] R.H. Law, N. Lukoyanova, I. Voskoboinik, T.T. Caradoc-Davies, K. Baran, M.A. Dunstone, M.E. D'Angelo, E.V. Orlova, F. Coulibaly, S. Verschoor, K.A. Browne, A. Ciccone, M.J. Kuiper, P.I. Bird, J.A. Trapani, H.R. Saibil, J.C. Whisstock, The structural basis for membrane binding and pore formation by lymphocyte perforin, Nature, 468 (2010) 447-451.

[87] J. Rossjohn, S.C. Feil, W.J. McKinstry, R.K. Tweten, M.W. Parker, Structure of a cholesterol-binding, thiol-activated cytolysin and a model of its membrane form, Cell, 89 (1997) 685-692.

[88] E. Mulvihill, K. van Pee, S.A. Mari, D.J. Muller, O. Yildiz, Directly Observing the Lipid-Dependent Self-Assembly and Pore-Forming Mechanism of the Cytolytic Toxin Listeriolysin O, Nano Lett, (2015).

[89] S.E. Stewart, M.E. D'Angelo, S. Paintavigna, R.F. Tabor, L.L. Martin, P.I. Bird, Assembly of streptolysin O pores assessed by quartz crystal microbalance and atomic force microscopy provides evidence for the formation of anchored but incomplete oligomers, Biochim Biophys Acta, 1848 (2015) 115-126.

[90] L.H. Young, S.V. Joag, L.M. Zheng, C.P. Lee, Y.S. Lee, J.D. Young, Perforin-mediated myocardial damage in acute myocarditis, Lancet, 336 (1990) 1019-1021.

[91] A.J. Wallace, T.J. Stillman, A. Atkins, S.J. Jamieson, P.A. Bullough, J. Green, P.J. Artymiuk, E. coli hemolysin E (HlyE, ClyA, SheA): X-ray crystal structure of the toxin and observation of membrane pores by electron microscopy, Cell, 100 (2000) 265-276.

[92] J.M. Mancheno, J. Martin-Benito, M. Martinez-Ripoll, J.G. Gavilanes, J.A. Hermoso, Crystal and electron microscopy structures of sticholysin II actinoporin reveal insights into the mechanism of membrane pore formation, Structure, 11 (2003) 1319-1328.

[93] K. Tanaka, J.M. Caaveiro, K. Morante, J.M. Gonzalez-Manas, K. Tsumoto, Structural basis for self-assembly of a cytolytic pore lined by protein and lipid, Nat Commun, 6 (2015) 6337.

[94] D. Westphal, R.M. Kluck, G. Dewson, Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis, Cell Death Diff, 21 (2014) 196-205.

[95] S.W. Muchmore, M. Sattler, H. Liang, R.P. Meadows, J.E. Harlan, H.S. Yoon, D. Nettesheim, B.S. Chang, C.B. Thompson, S.L. Wong, S.L. Ng, S.W. Fesik, X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death, Nature, 381 (1996) 335-341.

[96] E. Pays, B. Vanhollebeke, P. Uzureau, L. Lecordier, D. Perez-Morga, The molecular arms race between African trypanosomes and humans, Nat Rev Microbiol, 12 (2014) 575-584.

[97] A.J. Garcia-Saez, M. Coraiola, M.D. Serra, I. Mingarro, P. Muller, J. Salgado, Peptides corresponding to helices 5 and 6 of Bax can independently form large lipid pores, FEBS J, 273 (2006) 971-981.

[98] A.A. Sobko, E.A. Kotova, Y.N. Antonenko, S.D. Zakharov, W.A. Cramer, Effect of lipids with different spontaneous curvature on the channel activity of colicin E1: evidence in favor of a toroidal pore, FEBS Lett, 576 (2004) 205-210.

[99] S. Qian, W. Wang, L. Yang, H.W. Huang, Structure of transmembrane pore induced by Bax-derived peptide: evidence for lipidic pores, Proc Nat'l Acad Sci USA, 105 (2008) 17379-17383.

[100] M.G. Annis, E.L. Soucie, P.J. Dlugosz, J.A. Cruz-Aguado, L.Z. Penn, B. Leber, D.W. Andrews, Bax forms multispanning monomers that oligomerize to permeabilize membranes during apoptosis, EMBO J, 24 (2005) 2096-2103.

[101] D. Westphal, G. Dewson, M. Menard, P. Frederick, S. Iyer, R. Bartolo, L. Gibson, P.E. Czabotar, B.J. Smith, J.M. Adams, R.M. Kluck, Apoptotic pore formation is associated with in-plane insertion of Bak or Bax central helices into the mitochondrial outer membrane, Proc Nat'l Acad Sci USA, 111 (2014) E4076-4085.

[102] G.N. Moll, W.N. Konings, A.J. Driessen, Bacteriocins: mechanism of membrane insertion and pore formation, Antonie van Leeuwenhoek, 76 (1999) 185-198.

[103] S.D. Zakharov, E.A. Kotova, Y.N. Antonenko, W.A. Cramer, On the role of lipid in colicin pore formation, Biochim Biophys Acta, 1666 (2004) 239-249.

[104] X.P. Xu, D. Zhai, E. Kim, M. Swift, J.C. Reed, N. Volkmann, D. Hanein, Three-dimensional structure of Bax-mediated pores in membrane bilayers, Cell Death Dis, 4 (2013) e683.

[105] P.E. Czabotar, D. Westphal, G. Dewson, S. Ma, C. Hockings, W.D. Fairlie, E.F. Lee, S. Yao, A.Y. Robin, B.J. Smith, D.C. Huang, R.M. Kluck, J.M. Adams, P.M. Colman, Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis, Cell, 152 (2013) 519-531.

[106] S. Bleicken, O. Landeta, A. Landajuela, G. Basanez, A.J. Garcia-Saez, Proapoptotic Bax and Bak proteins form stable protein-permeable pores of tunable size, J Biol Chem, 288 (2013) 33241-33252.

[107] S. Bleicken, C. Wagner, A.J. Garcia-Saez, Mechanistic differences in the membrane activity of Bax and Bcl-xL correlate with their opposing roles in apoptosis, Biophys J, 104 (2013) 421-431.

[108] Y. Subburaj, K. Cosentino, M. Axmann, E. Pedrueza-Villalmanzo, E. Hermann, S. Bleicken, J. Spatz, A.J. Garcia-Saez, Bax monomers form dimer units in the membrane that further self-assemble into multiple oligomeric species, Nat Commun, 6 (2015) 8042.

[109] R. Amino, D. Giovannini, S. Thiberge, P. Gueirard, B. Boisson, J.F. Dubremetz, M.C. Prevost, T. Ishino, M. Yuda, R. Menard, Host cell traversal is important for progression of the malaria parasite through the dermis to the liver, Cell Host Microbe, 3 (2008) 88-96.

[110] S.C. Harrison, Viral membrane fusion, Nat Struct Mol Biol, 15 (2008) 690-698.

[111] S. Martens, H.T. McMahon, Mechanisms of membrane fusion: disparate players and common principles, Nat Rev Mol Cell Biol, 9 (2008) 543-556.

[112] S. Morlot, A. Roux, Mechanics of dynamin-mediated membrane fission, Ann Rev Biophys, 42 (2013) 629-649.

[113] O. Daumke, A. Roux, V. Haucke, BAR domain scaffolds in dynamin-mediated membrane fission, Cell, 156 (2014) 882-892.

[114] W. Almers, Fusion needs more than SNAREs, Nature, 409 (2001) 567-568.

[115] C. Peters, M.J. Bayer, S. Buhler, J.S. Andersen, M. Mann, A. Mayer, Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion, Nature, 409 (2001) 581-588.

[116] A.J. Merz, What are the roles of V-ATPases in membrane fusion?, Proc Nat'l Acad Sci USA, 112 (2015) 8-9.

[117] B. Strasser, J. Iwaszkiewicz, O. Michielin, A. Mayer, The V-ATPase proteolipid cylinder promotes the lipid-mixing stage of SNARE-dependent fusion of yeast vacuoles, EMBO J, 30 (2011) 4126-4141.

[118] E. Evans, M. Metcalfe, Free energy potential for aggregation of mixed phosphatidylcholine/phosphatidylserine lipid vesicles in glucose polymer (dextran) solutions, Biophys J, 45 (1984) 715-720.

[119] D. Evans, D. Needham, Attraction between lipid bilayer membranes in concentrated solutions of nonadsorbing polymers: comparisonofmean-field theory with measurements of adhesion energy., Macromolecules, 21 (1988) 1822-1831.

[120] S. Igonet, F.A. Rey, SnapShot: Viral and eukaryotic protein fusogens, Cell, 151 (2012) 1634-1634 e1631.

[121] J. Kadlec, S. Loureiro, N.G. Abrescia, D.I. Stuart, I.M. Jones, The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines, Nat Struct Mol Biol, 15 (2008) 1024-1030.

[122] R. Jahn, D. Fasshauer, Molecular machines governing exocytosis of synaptic vesicles, Nature, 490 (2012) 201-207.

[123] X. Bian, R.W. Klemm, T.Y. Liu, M. Zhang, S. Sun, X. Sui, X. Liu, T.A. Rapoport, J. Hu, Structures of the atlastin GTPase provide insight into homotypic fusion of endoplasmic reticulum membranes, Proc Nat'l Acad Sci USA, 108 (2011) 3976-3981.

[124] L.J. Byrnes, H. Sondermann, Structural basis for the nucleotide-dependent dimerization of the large G protein atlastin-1/SPG3A, Proc Nat'l Acad Sci USA, 108 (2011) 2216-2221.

[125] A.M. van der Bliek, Q. Shen, S. Kawajiri, Mechanisms of mitochondrial fission and fusion, Cold Spring Harbor Perspectives in Biology, 5 (2013).

[126] J. Perez-Vargas, T. Krey, C. Valansi, O. Avinoam, A. Haouz, M. Jamin, H. Raveh-Barak, B. Podbilewicz, F.A. Rey, Structural basis of eukaryotic cell-cell fusion, Cell, 157 (2014) 407-419.

[127] N.G. Abrescia, D.H. Bamford, J.M. Grimes, D.I. Stuart, Structure unifies the viral universe, Ann Rev Biochem, 81 (2012) 795-822.

[128] G. Anderluh, M. Kisovec, N. Krasevec, R.J. Gilbert, Distribution of MACPF/CDC Proteins, Sub-cell Biocem, 80 (2014) 7-30.

[129] D.H. Bamford, J.M. Grimes, D.I. Stuart, What does structure tell us about virus evolution?, Curr Op Struct Biol, 15 (2005) 655-663.

[130] R.J.C. Gilbert, Structural Features of Cholesterol Dependent Cytolysins and Comparison to other MACPF-domain containing proteins, in: G. Anderluh, R.J.C. Gilbert (Eds.) MACPF/CDC proteins - Agents of Defence, Attack and Invasion, Springer, Dordrecht/NL, 2014.

[131] J. Ravantti, D. Bamford, D.I. Stuart, Automatic comparison and classification of protein structures, J Biol Chem, 183 (2013) 47-56.

[132] L. De Colibus, A.F. Sonnen, K.J. Morris, C.A. Siebert, P. Abrusci, J. Plitzko, V. Hodnik, M. Leippe, E. Volpi, G. Anderluh, R.J. Gilbert, Structures of lysenin reveal a shared evolutionary origin for pore-forming proteins and its mode of sphingomyelin recognition, Structure, 20 (2012) 1498-1507.

[133] S. Roche, S. Bressanelli, F.A. Rey, Y. Gaudin, Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G, Science, 313 (2006) 187-191.

[134] P.A. Bullough, F.M. Hughson, J.J. Skehel, D.C. Wiley, Structure of influenza haemagglutinin at the pH of membrane fusion, Nature, 371 (1994) 37-43.

[135] G. Anderluh, R.J.C. Gilbert, MACPF/CDC Proteins - Agents of Defence, Attack and Invasion, Springer, Dordrecht, NL, 2014.

[136] H.R. Marsden, I. Tomatsu, A. Kros, Model systems for membrane fusion, Chemical Society reviews, 40 (2011) 1572-1585.

[137] H.J. Risselada, C. Kutzner, H. Grubmuller, Caught in the act: visualization of SNARE-mediated fusion events in molecular detail, Chembiochem, 12 (2011) 1049-1055.

[138] Y. Modis, S. Ogata, D. Clements, S.C. Harrison, Structure of the dengue virus envelope protein after membrane fusion, Nature, 427 (2004) 313-319.

[139] D.I. Stuart, M. Levine, H. Muirhead, D.K. Stammers, Crystal structure of cat muscle pyruvate kinase at a resolution of 2.6 A, J Mol Biol, 134 (1979) 109-142.

[140] N. Riffel, K. Harlos, O. Iourin, Z. Rao, A. Kingsman, D. Stuart, E. Fry, Atomic resolution structure of Moloney murine leukemia virus matrix protein and its relationship to other retroviral matrix proteins, Structure, 10 (2002) 1627-1636.

[141] J. Felsenstein, An alternating least squares approach to inferring phylogenies from pairwise distances, Syst Biol, 46 (1997) 101-111.